FILE 'CAPLUS' ENTERED AT 09:50:10 ON 05 JAN 2004

=> S TRANSGLUTAMINASE;S RECOMBINANT;S L1(3A)L2
         3958 TRANSGLUTAMINASE
          377 TRANSGLUTAMINASES
L1   4000 TRANSGLUTAMINASE
                (TRANSGLUTAMINASE OR TRANSGLUTAMINASES)

       151943 RECOMBINANT
         6220 RECOMBINANTS
L2   155371 RECOMBINANT
                (RECOMBINANT OR RECOMBINANTS)

L3   57 L1(3A)L2

=> D 1-57 CBIB ABS

L3 ANSWER 1 OF 57 CAPLUS COPYRIGHT 2004 ACS on STN
2003:719270 Document No. 139:244701 Cross-linked antigen vaccines for treating infections, cancers, autoimmune diseases and Alzheimer's diseases. Chou, Szu-yi (USA). PCT Int. Appl. WO 2003074004 A2 20030912, 130 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2003-US6661 20030303. PRIORITY: US 2002-PV361166 20020301; US 2002-PV363445 20020308; US 2002-231114 20020828; US 2002-231470 20020828; US 2002-231063 20020828; US 2002-231213 20020828; US 2002-231298 20020828.

AB Embodiments of the invention generally provide methods and compns. for producing polyvalent antigens, disease-specific antigens, ***transglutaminase*** -reactive compds., and ***recombinant*** ***transglutaminases*** . A method of producing a cross-linked compd. by a biol. agent is also provided. The invention provides a method for producing a cross-linked antigen and a method of using cross-linked products as antigens to immunize animals and induce strong immune responses. In addn., a method of producing an antigen specific for Alzheimer's disease is provided. Further, a method of producing a polyvalent antigen for two or more diseases is provided. Thus, compns. of antigens are prepd. and provided to immunize animals and induce strong immune responses. The invention provides purified ***recombinant*** ***transglutaminases*** reactive to a broad range of compds. and exhibit broad substrate activity. The ***recombinant*** ***transglutaminases*** are ***recombinant*** or chimeric ***transglutaminases*** of Streptomyces mobaraensis or Streptomyces cinnamoneus. Addnl., the invention provides a method of attaching one or more amino acid residues to a compd. to be reactive with transglutaminase, even for transglutaminase non-reactive compds.

L3 ANSWER 2 OF 57 CAPLUS COPYRIGHT 2004 ACS on STN
2003:298379 Document No. 139:178939 Human ***recombinant*** anti- ***transglutaminase*** antibody testing is useful in the diagnosis of silent coeliac disease in a selected group of at-risk patients. Vivas, Santiago; Ruiz de Morales, Jose M.; Martinez, Julio; Gonzalez, Maria Cruz; Martin, Sara; Martin, Juan; Cechini, Carlos; Olcoz, Jose Luis (Gastroenterology Units, Hospital de Leon, Leon, Spain). European Journal of Gastroenterology & Hepatology, 15(5), 479-483 (English) 2003. CODEN: EJGHES. ISSN: 0954-691X. Publisher: Lippincott Williams & Wilkins.

AB Functional dyspepsia, unexplained chronic hypertransaminasemia (CHT) and hepatitis C virus (HCV) are common gastrointestinal situations that have been related to celiac disease. Antibodies to tissue transglutaminase (tTG) have been claimed recently to be highly effective as a screening method for celiac disease. The objective was to assess the prevalence of celiac disease by means of detection of antibodies against human tTG in the above-mentioned groups of patients. A control group consisted of 165

normal blood donors. Patient groups comprised 90 CHT patients, 102 HCV patients and 92 functional dyspepsia patients. All patients were tested for anti-tTG (IgA) antibodies. Anti-endomysium (IgA) antibodies (AEA) and antigliadin (IgA) antibodies (AGA) and antigliadin (IgG) antibodies (AGG) were also tested. When anti-tTG or AEA was pos., a duodenal biopsy was recommended. One of 165 blood donors, three of 92 functional dyspepsia patients, four of 90 CHT patients and none of 102 HCV patients were pos. for anti-tTG antibodies. In the anti-tTG-pos. group, all but one were AEA-pos. There were no AEA- or AGA IgA-positives that revealed a neg. anti-tTG test. Duodenal biopsy confirmed a diagnosis of celiac disease in all the cases. Statistically significant differences were found between the controls and the functional dyspepsia group and between the controls and the CHT group, but not between the controls and the HCV group. Both CHT and functional dyspepsia may represent a true oligosymptomatic form of celiac disease. In such conditions, the detection of anti-tTG antibodies is useful as a screening method. Celiac disease is not an autoimmune manifestation of HCV, so screening for celiac disease in HCV patients cannot be recommended.

L3 ANSWER 3 OF 57 CAPLUS COPYRIGHT 2004 ACS on STN

2003:251492 Document No. 139:242089 N-terminus end of rat prostate transglutaminase is responsible for its catalytic activity and GTP binding. Mariniello, Loredana; Esposito, Carla; Caputo, Ivana; Sorrentino, Angela; Porta, Raffaele (Department of Food Science, University of Naples "Federico II", Naples, 80055, Italy). International Journal of Biochemistry & Cell Biology, 35(7), 1098-1108 (English) 2003. CODEN: IJBBFU. ISSN: 1357-2725. Publisher: Elsevier Science Ltd..

AB Rat prostate transglutaminase is characterized by a high degree of complexity. In fact, as previously demonstrated, it is highly glycosylated and possesses a lipid anchor which is retained during enzyme apocrine secretion. In order to assess the importance of such modifications upon enzyme functionality, full length rat prostate transglutaminase cDNA has been synthesized by RT-PCR and stably expressed in MDCK cells. The recombinant form has been partially purified by GTP-affinity chromatog., a technique which has been used to purify the enzyme produced from rat prostate secretion. The recombinant protein is endowed with enzymic activity even though, as we have demonstrated by immunol. studies, it lacks post-translational modifications which occur in the prostate enzyme. Moreover, we have demonstrated that a deletion mutant, which gives rise to a protein lacking 103 amino acid residues at the N-terminus end, loses enzymic activity and the capability of binding GTP. This study shows that, while post-translational modifications are not essential for enzymic activity, the N-terminus end is responsible for both transglutaminase functionality and GTP-binding.

L3 ANSWER 4 OF 57 CAPLUS COPYRIGHT 2004 ACS on STN

2003:246116 Document No. 138:352149 High rate of positive anti-tissue transglutaminase antibodies in chronic liver disease: Role of liver decompensation and of the antigen source. Vecchi, M.; Folli, C.; Donato, M. F.; Formenti, S.; Arosio, E.; de Franchis, R. (Gastroenterology Unit, Dept. of Internal Medicine and Liver Unit, IRCCS Policlinico Hospital and University of Milan, Italy). Scandinavian Journal of Gastroenterology, 38(1), 50-54 (English) 2003. CODEN: SJGRA4. ISSN: 0036-5521. Publisher: Taylor & Francis.

AB Background: Since the recognition of tissue transglutaminase (tTG) as the target antigen of anti-endomysium antibodies, several ELISA assays using either guinea pig or human recombinant tTG have been developed. The aim of the study was to compare the behavior of anti-tTG and anti-endomysium antibodies assays in celiacs and in patients with chronic liver disease. Methods: 34 patients (24 women, 34.9.+-.12.5 yr) with celiac disease and 41 with chronic liver disease (14 women, 57.+-.11.2 yr), including 19 cirrhotics, were evaluated for anti-endomysium antibodies by indirect immunofluorescence and for anti-tTG IgA antibodies by ELISA, using guinea pig liver or human ***recombinant*** ***transglutaminase*** . Results: The prevalences of anti-tTG and anti-endomysium antibodies were 100% in patients with celiac disease at diagnosis, 75% and 64.3% in patients on a gluten-free diet. All liver disease patients were neg. for anti-endomysium antibodies, while 11 (26.8%) were pos. for anti-tTG. All these patients had liver cirrhosis and represented 57.9% of all cirrhotics. The presence of anti-tTG was assocd. with higher Child-Pugh scores. The use of human transglutaminase detd. a redn. in the rate of

pos. results; however, the rate of pos. anti-tTG was still 17.1% in all liver disease patients and 31.6% in cirrhotics. Conclusions: Our data confirm that anti-tTG have a similar sensitivity compared with anti-endomysium antibodies assay in celiacs. However, a high prevalence of pos. anti-tTG results is obsd. in cirrhotic patients, even when human recombinant tTG is used. The high prevalence of pos. results among cirrhotic patients is assocd. with more advanced liver disease.

L3 ANSWER 5 OF 57 CAPLUS COPYRIGHT 2004 ACS on STN

2003:222257 Document No. 138:249930 Cloning of the cDNA for human transglutaminase ztg2 and use thereof. Fox, Brian A. (USA). U.S. Pat. Appl. Publ. US 2003054526 A1 20030320, 38 pp. (English). CODEN: USXXCO. APPLICATION: US 2002-109084 20020327. PRIORITY: US 2001-PV279289 20010328.

AB Transglutaminase polypeptides, polynucleotides encoding them, methods of making them, and methods of using them are disclosed. Specifically, the cDNA and protein sequences for a human transglutaminase ztg2 704-amino acid C-terminal fragment are provided. Also disclosed are methods and vectors for ***recombinant*** ***transglutaminase*** ztg2 expression, which might be useful for forming an .epsilon.-(.gamma.-glutaminyl)lysine isopeptide bond between two proteins. The compns. and methods of the invention may be used for a variety of purposes in industry, research, and medicine.

L3 ANSWER 6 OF 57 CAPLUS COPYRIGHT 2004 ACS on STN

2003:193524 Document No. 138:333610 Structure of folding intermediates at pH 4.0 and native state of microbial transglutaminase. Yokoyama, Kei-Ichi; Ejima, Daisuke; Kita, Yoshiko; Philo, John S.; Arakawa, Tsutomu (Central Research Laboratories, Ajinomoto Co., Inc., Kawasaki, 210-8681, Japan). Bioscience, Biotechnology, and Biochemistry, 67(2), 291-294 (English) 2003. CODEN: BBBIEJ. ISSN: 0916-8451. Publisher: Japan Society for Bioscience, Biotechnology, and Agrochemistry.

AB Recombinant Streptoverticillium sp. strain s-8112 transglutaminase was expressed in Escherichia coli as insol. inclusion bodies. After the authors searched for refolding conditions, it was found that refolding of the protein could be performed 1st by diln. of the unfolded enzyme in a buffer at pH 4.0, and then by titrn. of the pH from 4.0 to 6.0. CD anal. showed that a burst of secondary structure formation occurred within the dead time of the expt. and accounted for 75% of the signal change in the far-UV CD, with little tertiary structure being formed. This burst was followed by slow rearrangement of the secondary structure accompanied by the formation of tertiary structure. The secondary and tertiary structures of the final sample at pH 4.0, corresponding to the folding intermediate, were different from these structures at pH 6.0. Once the native structure was obtained, acidification of the native protein to pH 4.0 did not lead to a structure like that of the folding intermediate. Sedimentation velocity anal. showed that the folding intermediate had an expanded structure and contained no other structure species including large aggregates.

L3 ANSWER 7 OF 57 CAPLUS COPYRIGHT 2004 ACS on STN

2003:137405 Document No. 138:320006 Gelation of food protein induced by ***recombinant*** microbial ***transglutaminase*** . Yokoyama, K.; Ohtsuka, T.; Kuraishi, C.; Ono, K.; Kita, Y.; Arakawa, T.; Ejima, D. (Central Research Laboratories, Ajinomoto Co., Inc., Kanagawa, 210-8681, Japan). Journal of Food Science, 68(1), 48-51 (English) 2003. CODEN: JFDSAZ. ISSN: 0022-1147. Publisher: Institute of Food Technologists.

AB The ***recombinant*** microbial ***transglutaminase*** from Streptoverticillium mobaraense var. (rMTGase) was expressed in Escherichia coli. Specific enzyme activity of rMTGase was comparable to native MTGase. However, the gelation of a sodium caseinate soln. induced by rMTGase was slower than that induced by native MTGase. In addn., the mech. property of kamaboko prepd. with rMTGase was weaker than that with native MTGase. In SDS-PAGE anal., .alpha.-casein monomers decreased more slowly during the incubation with rMTGase than MTGase. These results confirmed the difference of crosslinking activity between the 2 enzymes. Furthermore, thermal stability of rMTGase was lower compared to native MTGase. These results suggest that the difference of crosslinking activity and thermal stability between the 2 enzymes cause differences in gelation activity of protein.

L3 ANSWER 8 OF 57 CAPLUS COPYRIGHT 2004 ACS on STN

2003:76914 Document No. 138:132149 Promoter DNA sequences for regulating transcription of genes for recombinant enzyme production in fungi. Lehmbeck, Jan (Novozymes A/s, Den.). PCT Int. Appl. WO 2003008575 A2 20030130, 48 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2002-DK500 20020718. PRIORITY: DK 2001-1132 20010720.

AB The invention claims a DNA cassette for regulating transcription of a structural gene encoding a polypeptide in a eukaryotic host cell comprising (i) a first DNA sequence to which RNA polymerase binds which DNA sequence comprises a mRNA initiation site; and (ii) one or more DNA sequence(s) to which RNA polymerase binds with or without a mRNA initiation site. Specifically, the invention claims a DNA sequence comprising the Aspergillus niger neutral amylase 2 (NA2) promoter fused with the Aspergillus nidulans triose phosphate isomerase leader sequence. The invention also claims DNA constructs with two or more tandem repetitive RNA polymerase binding sites and use of the Aspergillus amyR transcription factor binding site. In addn., the DNA construct is claimed for increasing transcription of genes encoding enzymes including oxidoreductase, transferase, hydrolase, lyase, isomerase, glucoamylase, and more. The invention also claims DNA clones, expression vectors, and host cells contg. DNA sequences of the invention. Examples of the invention describe cloning of a no. of plasmid vectors contg. the Aspergillus niger neutral amylase 2 (NA2) promoter, construction and genetic transformation of Aspergillus oryzae or Aspergillus niger host strains, and lipase or pectin Me esterase expression in transformants.

L3 ANSWER 9 OF 57 CAPLUS COPYRIGHT 2004 ACS on STN

2002:903486 Document No. 138:121824 Microbial ***transglutaminase*** and ***recombinant*** cystatin effects on improving the quality of mackerel surimi. Hsieh, J.-F.; Tsai, G.-J.; Jiang, S.-T. (Dept. of Food Science, National Taiwan Ocean Univ., Chi-lung, 202, Taiwan). Journal of Food Science, 67(8), 3120-3125 (English) 2002. CODEN: JFDSAZ. ISSN: 0022-1147. Publisher: Institute of Food Technologists.

AB Effects of microbial ***transglutaminase*** (MTGase), ***recombinant*** cystatin, or their combination on the gel properties of mackerel surimi were investigated. The addn. of MTGase caused the crosslinking of myosin heavy chain (MHC) and substantially increased the gel strength (from 536.6 to 2012.4 g .times. cm), while the recombinant cystatin could effectively prevent the MHC degrdn. and gel softening during the prodn. of mackerel surimi-based products. Combined use of MTGase and recombinant cystatin revealed synergistic effectiveness on improving the quality of mackerel surimi (increased from 435 to 2438 g .times. cm, set at 45.degree. for 20 min).

L3 ANSWER 10 OF 57 CAPLUS COPYRIGHT 2004 ACS on STN

2002:883180 Document No. 138:283105 Production of ***recombinant*** human tissue ***transglutaminase*** using the baculovirus expression system, and its application for serological diagnosis of coeliac disease. Osman, Awad A.; Richter, Thomas; Stern, Martin; Conrad, Karsten; Henker, Jobst; Brandsch, Corinna; Zimmer, Klaus-Peter; Mothes, Thomas (Institute of Laboratory Medicine, Clinical Chemistry, and Molecular Diagnostics and Department of Paediatrics, University Hospital, Leipzig, Germany). European Journal of Gastroenterology & Hepatology, 14(11), 1217-1223 (English) 2002. CODEN: EJGHES. ISSN: 0954-691X. Publisher: Lippincott Williams & Wilkins.

AB Tissue transglutaminase was identified as the main autoantigen in coeliac disease (CD) but enzyme immunoassays applying the com. available antigen from guinea pig liver show insufficient specificity and sensitivity for diagnosis as compared with endomysium antibodies (EmA). The aim of this present study was to develop a new method for the cloning and expression of human tissue transglutaminase (hu-tTG) and to test hu-tTG in the serol. diagnosis of CD. Hu-tTG was cloned and expressed using a baculovirus system and SF9 insect cells. The enzyme carried a C-terminal His tag

allowing efficient affinity purifn. from cell lysates. The recognition of hu-tTG by human sera was checked by using an enzyme linked immunosorbent assay (ELISA). For this, 35 patients with active CD were compared with 144 controls (18 patients with bioptically excluded CD, 89 blood donors, 30 patients with inflammatory bowel disease, and seven patients with cystic fibrosis). The ELISA using hu-tTG showed a sensitivity of 100% and a specificity of 98.6%. Titers of antibodies against hu-tTG (anti-hu-tTG) were pos. correlated with EmA titers. All results neg. for EmA were also neg. for anti-hu-tTG. There were, however, EmA pos. up to a titer of 1:80 below the cut-off for anti-hu-tTG. For comparison, antibodies against guinea pig tissue transglutaminase (anti-gp-tTG) were detd. in parallel. All patients with anti-hu-tTG below the cut-off were also neg. for anti-gp-tTG. However, there were eight patients pos. for anti-hu-tTG but neg. for anti-gp-tTG. The new test reaches and even exceeds diagnostic efficiency of EmA for coeliac diagnosis.

L3 ANSWER 11 OF 57 CAPLUS COPYRIGHT 2004 ACS on STN

2002:796175 Document No. 138:135603 Bone-Specific Antibodies in Sera from Patients with Celiac Disease: Characterization and Implications in Osteoporosis. Sugai, Emilia; Chernavsky, Alejandra; Pedreira, Silvia; Smecuol, Edgardo; Vazquez, Horacio; Niveloni, Sonia; Mazure, Roberto; Maurino, Eduardo; Rabinovich, Gabriel A.; Bai, Julio C. (School of Medicine, Gastroenterology Department, Universidad del Salvador, Buenos Aires, Argent.). Journal of Clinical Immunology, 22(6), 353-362 (English) 2002. CODEN: JCIMDO. ISSN: 0271-9142. Publisher: Kluwer Academic/Plenum Publishers.

AB Osteopenia and osteoporosis are well-known complications detected in celiac disease patients with still obscure pathogenesis. In the present study we investigated the presence of circulating anti-bone autoantibodies in patients with celiac disease and explored their role in the assocd. bone disease. We evaluated serum samples from 33 patients at the time of diagnosis and from 20 of them after treatment. Sera from patients with inflammatory bowel disease (n = 9), nonceliac osteoporotic (n = 18), and healthy individuals (n = 10) were used as controls. The presence of IgA specific anti-bone antibodies was first investigated using indirect immunofluorescence on cryosections of fetal rat tibia (20-day pregnancy). Furthermore, samples were homogenized and total tissue exts. were subjected to Western blot anal. to confirm immunoreactivity. At diagnosis, sera from 51.5% (17/33) of celiac patients had antibodies that recognized antigenic structures in chondrocytes and the extracellular matrix along mature cartilage, bone interface, and perichondrium of fetal rat bone. Among controls, only two osteoporotic patients showed very low titers of anti-bone autoantibodies. The immunostaining was localized in areas where an active mineralization process occurred and was similar to the distribution of the native bone tissue transglutaminase. The frequency of patients with pos. baseline titers of anti-bone antibodies diminished significantly after treatment (P = 0.048). Western blot assays confirmed the presence of autoantibodies in sera from patients with a pos. immunofluorescence staining. Autoantibodies recognized a major protein band on tissue exts. with a mol. wt. of 77-80 kDa, which could be displaced when sera were preadsorbed with human ***recombinant*** tissue ***transglutaminase*** . We provide original evidence that patients with celiac disease have IgA-type circulating autoantibodies against intra- and extracellular structures of fetal rat tibia. Our findings suggest that these antibodies recognize bone tissue transglutaminase as the autoantigen, and based on the localization of the immunoreactivity we speculate that they might have an active role in the pathophysiol. of celiac disease-assocd. bone complications.

L3 ANSWER 12 OF 57 CAPLUS COPYRIGHT 2004 ACS on STN

2002:596669 Document No. 137:336399 Antibodies against human tissue transglutaminase and endomysium in diagnosing and monitoring coeliac disease. Buergin-Wolff, A.; Dahlbom, I.; Hadziselimovic, F.; Petersson, C. J. (Institute for Coeliac Disease, Liestal, Switz.). Scandinavian Journal of Gastroenterology, 37(6), 685-691 (English) 2002. CODEN: SJGRA4. ISSN: 0036-5521. Publisher: Taylor & Francis.

AB Background: Coeliac disease (CD) patients often present a variety of uncharacteristic symptoms and therefore sensitive and specific screening tests are needed as an aid in making an accurate diagnosis. A recently developed ELISA, using human ***recombinant*** tissue ***transglutaminase*** (tTG) as antigen, was evaluated for its

significance in the diagnosis of CD. The patient's compliance to a gluten-free diet and the serol. reaction during gluten challenge were also monitored. The results were compared with IgA-endomysium antibody (EMA) results. Methods: Sera previously collected from 365 patients (0.4-76 yr) with jejunal biopsy on a gluten-contg. diet and from 41 patients on a gluten-free diet or challenge were tested for IgA anti-human tTG antibodies (IgA tTG ab) with Celikey (Pharmacia Diagnostics). The study population comprised 208 CD patients and 157 controls. The diagnostic performance and cut-off for the assay were estd. with ROC anal. EMA was analyzed by indirect immunofluorescence microscopy on cryostat sections of monkey esophagus. Results: 200/208 patients with CD had pos. IgA tTG ab (median >100 U/mL), while only 1/157 of the control patients were pos. (median 1.67 U/mL). The area under the ROC curve was 98.3% and the sensitivity and specificity of the test were 96% and 99% for the study population. Only 4/365 patients (1%) presented discordant IgA tTG ab and EMA results, 2 of them had only IgA tTG ab and 2 only EMA. The IgA tTG ab levels and the EMA titers were closely correlated to the duration of gluten-free diet and gluten challenge, resp. Conclusion: IgA tTG ab can be used as an accurate observer-independent alternative to EMA in diagnosing or monitoring CD.

L3 ANSWER 13 OF 57 CAPLUS COPYRIGHT 2004 ACS on STN

2002:550939 Document No. 137:105592 Characterization of ***recombinant*** ***transglutaminases*** 1 and 3 expressed in baculovirus system. Hitomi, Kiyotaka; Ikura, Koji; Maki, Masatoshi (Laboratory of Molecular and Cellular Regulation, Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya, 464-8601, Japan). Animal Cell Technology: Basic & Applied Aspects, Proceedings of the Annual Meeting of the Japanese Association for Animal Cell Technology, 13th, Fukuoka and Karatsu, Japan, Nov. 16-21, 2000, Meeting Date 2000, 167-171. Editor(s): Shirahata, Sanetaka; Teruya, Kiichiro; Katakura, Yoshinori. Kluwer Academic Publishers: Dordrecht, Neth. ISBN: 1-4020-0271-8 (English) 2002. CODEN: 69CWTU.

AB Transglutaminase (TGase) is a Ca2+-dependent enzyme that catalyze the formation of isopeptide crosslinks in proteins between the .gamma.-carboxamide groups of glutamine residues and .epsilon.-NH2 groups of Lys residues. Both TGases, keratinocyte-type (human TGase 1) and epidermal-type (mouse TGase 3) enzymes, are involved in the formation of the cornified cell envelope, which serves a vital barrier function for the skin, by crosslinking of a variety of structural proteins in the epidermis. The mol. wts. of TGases 1 and 3 were 107 and 77 kDa, resp. Both enzymes were activated by limited proteolysis during epidermal differentiation. It has been difficult to isolate sufficient quantities of native enzymes from tissues or to obtain recombinant proteins in a bacterial expression system for biochem. studies of TGase properties. Therefore, the authors circumvented these problems by expressing recombinant full-length cDNAs of the TGases in baculovirus-infected insect cells. The expressed proteins were purified to homogeneity by successive chromatog. and HPLC. Calpain, that had been a candidate for an activating enzyme, was not effective for the activation of TGase 3 zymogen. In the case of TGase 1, the calpain-proteolyzed form was highly sensitive to Ca2+ for TGase activity.

L3 ANSWER 14 OF 57 CAPLUS COPYRIGHT 2004 ACS on STN

2002:523572 Document No. 137:289959 Human transglutaminase sequence homolog, protein and cDNA sequences, recombinant production and therapeutic uses. Mao, Yumin; Xie, Yi (Bode Gene Development Co., Ltd., Shanghai, Peop. Rep. China). Faming Zhuanli Shenqing Gongkai Shuomingshu CN 1321670 A 20011114, 34 pp. (Chinese). CODEN: CNXXEV. APPLICATION: CN 2000-115542 20000429.

AB The invention relates to a human transglutaminase sequence homolog, designated as transglutaminase active site-contg. protein 9. The open reading frame of the cDNA encodes a protein with 80 amino acids, and an estd. mol. wt. of 9 kilodalton based on SDS-PAGE. The invention provides the use of polypeptide and polynucleotide in a method for treatment of various kinds of diseases, such as cancer, HIV, inflammation, immune disease, blood disease, metabolic disorder, growth disease and bleeding. The invention also relates to methods, expression vectors and host cells for ***recombinant*** prodn. of said ***transglutaminase*** sequence homolog. The invention also relates to agonist and antagonist of said transglutaminase sequence homolog and uses in therapy.

L3 ANSWER 15 OF 57 CAPLUS COPYRIGHT 2004 ACS on STN
2002:488472 Document No. 137:277004 ***Recombinant*** human tissue ***transglutaminase*** for diagnosis and follow-up of childhood coeliac disease. Hansson, Tony; Dahlbom, Ingrid; Rogberg, Siv; Dannaeus, Anders; Hopfl, Peter; Gut, Heidi; Kraaz, Wolfgang; Klareskog, Lars (Department of Rheumatology, Karolinska Hospital, Stockholm, SE-171 76, Swed.). Pediatric Research, 51(6), 700-705 (English) 2002. CODEN: PEREBL. ISSN: 0031-3998. Publisher: Lippincott Williams & Wilkins.

AB Highly discriminatory markers for celiac disease are needed to identify children with early mucosal lesions and for rapid follow-up. The aim of this study was to evaluate the potential of circulating anti-tissue transglutaminase (tTG) IgA and IgG antibodies in the diagnosis and follow-up of childhood celiac disease. An ELISA using recombinant human tTG was used to measure the levels of IgA and IgG anti-tTG antibodies in 226 serum samples from 57 children with biopsy-verified celiac disease, 29 disease control subjects, and 24 healthy control subjects. All samples were also analyzed for anti-endomysium antibodies (EMA). The levels of IgA and IgG anti-tTG antibodies correlated with the condition of the small intestinal villousv structure and the serum levels of IgA EMA. All of the 25 serum samples obtained from untreated patients contained IgA anti-tTG antibodies, and 24 of 25 also had IgA EMA. Of the serum samples from 53 control children, two had IgA anti-tTG antibodies and two had IgA EMA. Children younger than 5 y of age with untreated celiac disease had the highest serum levels of both IgA and IgG anti-tTG. There was already an increase in IgA anti-tTG antibodies after 2 wk of gluten challenge ($p < 0.01$). Although the criteria-based diagnosis of childhood celiac disease still depends on histol. evaluation of intestinal biopsies, detection of anti-tTG antibodies provides useful complementary diagnostic information. The human recombinant tTG-based ELISA can be used as a sensitive and specific test to support the diagnosis and may also be used in the follow-up of treatment in childhood celiac disease.

L3 ANSWER 16 OF 57 CAPLUS COPYRIGHT 2004 ACS on STN
2002:469164 Document No. 137:181442 Co-overexpression of folding modulators improves the solubility of the ***recombinant*** guinea pig liver ***transglutaminase*** expressed in Escherichia coli. Ikura, Koji; Kokubu, Tsuyoshi; Natsuka, Shunji; Ichikawa, Akira; Adachi, Motoyasu; Nishihara, Kazuyo; Yanagi, Hideki; Utsumi, Shigeru (Department of Applied Biology, Kyoto Institute of Technology, Kyoto, 606-8585, Japan). Preparative Biochemistry & Biotechnology, 32(2), 189-205 (English) 2002. CODEN: PBBIF4. ISSN: 1082-6068. Publisher: Marcel Dekker, Inc..

AB Transglutaminases (E.C. 2.3.2.13) catalyze the formation of .epsilon.-(.gamma.-glutamyl)lysine crosslinks and the substitution of primary amines for the .gamma.-carboxamide groups of protein-bound glutamine residues, and are involved in many biol. phenomena. Transglutaminase reactions are also applicable in applied enzymol. Here, we established an expression system of ***recombinant*** mammalian tissue-type ***transglutaminase*** with high productivity. Overexpression of guinea pig liver transglutaminase in Escherichia coli, using a plasmid pET21-d, mostly resulted in the accumulation of insol. and inactive enzyme protein. By the growing cells at lower temps. (25 and 18.degree.), however, a fraction of the sol. and active enzyme protein slightly increased. Co-overexpression of a mol. chaperone system (DnaK-DnaJ-GrpE) and/or a folding catalyst (trigger factor) improved the soly. of the recombinant enzyme produced in E. coli cells. The specific activity, the affinity to the amine substrate, and the sensitivity to the calcium activation and GTP inhibition of the purified sol. recombinant enzyme were lower than those of the natural liver enzyme. These results indicated that co-overexpression of folding modulators tested improved the soly. of the overproduced ***recombinant*** mammalian tissue-type ***transglutaminase*** , but the catalytic properties of the sol. recombinant enzyme were not exactly the same as those of the natural enzyme.

L3 ANSWER 17 OF 57 CAPLUS COPYRIGHT 2004 ACS on STN
2002:235424 Document No. 136:397703 Expression in Escherichia coli and purification of hexahistidine-tagged human tissue transglutaminase. Shi, Qingli; Kim, Soo-Youl; Blass, John P.; Cooper, Arthur J. L. (Department of Neurology and Neuroscience, Weil Medical College of Cornell University, New York, NY, 10021, USA). Protein Expression and Purification, 24(3),

366-373 (English) 2002. CODEN: PEXPEJ. ISSN: 1046-5928. Publisher: Elsevier Science.

AB Recent evidence suggests that aberrant transglutaminase activity is assocd. with a wide variety of diseases. Tissue transglutaminase (I) is the most widely distributed of the 6 well-characterized transglutaminases in humans. Here, the authors describe a method for expressing hexahistidine-tagged human I in Escherichia coli BL21(DE3) using the pET-30 Ek/LIC expression vector. The purifn. of the expressed enzyme from suspensions of E. coli cells treated with CelLytic B Bacterial Cell Lysis/Extn. Reagent was accomplished by immobilized metal (Ni2+) affinity column chromatog. The procedure typically yielded highly purified and highly active recombinant human I in .apprx.1 day (.apprx.0.6 mg/from 1 L culture). Conditions for stable storage of purified I were detd. as -80.degree. in Tris-acetate buffer (pH 8.0) contg. 0.5 mM EDTA and 10 mM dithiothreitol.

L3 ANSWER 18 OF 57 CAPLUS COPYRIGHT 2004 ACS on STN

2002:149344 Document No. 137:78031 Application of transglutaminase in seafood and meat processings. Jiang, Shann-Tzong; Yin, Li-Jung (Dep. Food Sci., National Taiwan Ocean Univ., Chi-lung, 202, Taiwan). Taiwan Shuichan Xuehuikan, 28(3), 151-162 (English) 2001. CODEN: TSCKD6. ISSN: 0379-4180. Publisher: Fisheries Society of Taiwan.

AB A review discussing microbial and ***recombinant*** ***transglutaminases*** and their application in seafood and meat processing. The modification of food proteins by transglutaminase may lead to textured products, help to protect lysine in food proteins from various chem. reactions, encapsulate the lipids and/or lipid-sol. materials, form heat- and water-resistant films, avoid heat treatment for gelation, improve elasticity and water-holding capacity, modify soly. and other functional properties, and produce food proteins with higher nutritive value through crosslinking of proteins.

L3 ANSWER 19 OF 57 CAPLUS COPYRIGHT 2004 ACS on STN

2002:142890 Document No. 136:196189 Method of modifying microbial transglutaminase substrate specificity by x-ray crystal structure-based designing and mutagenesis. Kashiwagi, Tatsuki; Shimba, Nobuhisa; Ishikawa, Kohki; Suzuki, Eiichiro; Yokoyama, Keiichi; Hirayama, Kazuo (Ajinomoto Co., Inc., Japan). PCT Int. Appl. WO 2002014518 A1 20020221, 126 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (Japanese). CODEN: PIXXD2. APPLICATION: WO 2001-JP7038 20010815. PRIORITY: JP 2000-247664 20000817; JP 2000-396695 20001227.

AB A method of designing and constructing a variant transglutaminase of Streptoverticillium mobaraense origin based on the three dimensional stereo-structure, for modifying substrate specificity and improving reactivity, is disclosed. The variant transglutaminase is designed and constructed by estg. the substrate-binding site of transglutaminase based on the stereo-structure obtained by anal. of the x-ray cryst. structure, and substituting, inserting or deleting the amino acid residue located at the substrate-binding site of the transglutaminase. Acidic amino acids, in particular, are deleted. Recombinant expression of the modified MTG. Transglutaminase crystals of the monoclinic system with P21 space group, are claimed. Expression of ***recombinant*** ***transglutaminase*** in E. coli and detn. of x-ray cryst. structure, are described. N-terminal Asp deletion mutant, mutants with Ser2 substituted with Tyr, Arg, or Asp, N-terminal 2 or 3 residues deletion mutants, mutants with residues 241-252 or 278-287 substituted with 3 Gly residues, were constructed.

L3 ANSWER 20 OF 57 CAPLUS COPYRIGHT 2004 ACS on STN

2002:89814 Document No. 136:139848 Linkage of agents using microparticles. Green, Howard; Compton, Bruce J.; Corey, George D.; Dijan, Philippe (Pericor Science, Inc., USA). PCT Int. Appl. WO 2002007707 A2 20020131, 91 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK,

LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2001-US22681 20010719. PRIORITY: US 2000-620783 20000721.

AB Methods, products and kits are provided for attaching active agents to a skin surface via microparticles using endogenous or exogenous transglutaminase. A microparticle comprises an active agent and a lysine or glutamine-rich polymer having transglutaminase substrate reactive groups. The microparticle further comprises a synthetic polymer, i.e., polystyrene, covalently linked to the amino acid-rich polymer. The microparticle is porous, non-biodegradable, and detergent insol. The active agent is selected from the group consisting of a cosmetic agent, a bulking agent, a hair conditioning agent, a hair fixative, a sunscreen agent, a moisturizing agent, a depilatory agent, an anti-nerve gas agent, a film forming agent, a vitamin, an insect repellent, a coloring agent, a pharmaceutical agent, a ligand-receptor complex and a receptor of a ligand-receptor complex. Microparticles are provided in a topically administered form, i.e., an ointment, an aerosol, a gel and a lotion. For example, a kit was provided for producing durable antifungal protection. The kit contained 3 components: Component 1 is a conjugate of an antifungal and a linking agent, i.e., an aq. soln., pH 6.4, contg. 0.01 wt.% polylysyl-amphotericin B conjugate, 10 vol.% ethanol, 0.1 vol.% propylene glycol, 0.5 mM EDTA, and 0.1 wt.% BHT. Component 2 is a calcium chloride activator soln., i.e., an aq. soln. contg. about 25 mM calcium chloride. Component 3 is a lyophilized transglutaminase prepn. contg. 10 mg of ***recombinant*** tissue ***transglutaminase*** in 2% sucrose, 0.1 mM EDTA, and 5 mM glycine buffer, pH 7.2. The three containers contg. components 1, 2 and 3 are opened and component 1 was mixed with component 3 and component 2 was then added.

L3 ANSWER 21 OF 57 CAPLUS COPYRIGHT 2004 ACS on STN

2001:792218 Document No. 135:343279 Parasitic nematode transglutaminase proteins and uses thereof. Chandrashekar, Ramaswamy; Mehta, Kapil (Heska Corp., USA; Board of Regents, the University of Texas System). U.S. US 6309644 B1 20011030, 66 pp., Cont.-in-part of U.S. 6,248,872. (English). CODEN: USXXAM. APPLICATION: US 1997-874102 19970612. PRIORITY: US 1996-781420 19961203.

AB The present invention relates to parasitic nematode transglutaminase proteins; to parasitic nematode transglutaminase nucleic acid mols., including those that encode such transglutaminase proteins; to antibodies raised against such transglutaminase proteins; and to compds. that inhibit parasitic nematode transglutaminase activity. The present invention also includes methods to obtain such proteins, nucleic acid mols., antibodies, and inhibitory compds. Also included in the present invention are therapeutic compns. comprising such proteins, nucleic acid mols., antibodies and/or inhibitory compds. as well as the use of such therapeutic compns. to protect animals from diseases caused by parasitic nematodes. This invention also relates to the surprising discovery that parasitic nematode transglutaminase proteins have protein disulfide isomerase activity. Accordingly, this invention relates further to inhibitors of the protein disulfide isomerase activity of said transglutaminases.

L3 ANSWER 22 OF 57 CAPLUS COPYRIGHT 2004 ACS on STN

2001:713394 Document No. 135:268281 Protein and cDNA sequences of 12 kDa human transglutaminase and therapeutic use thereof. Mao, Yumin; Xie, Yi (Shanghai Biowindow Gene Development Inc., Peop. Rep. China). PCT Int. Appl. WO 2001070787 A1 20010927, 37 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (Chinese). CODEN: PIXXD2. APPLICATION: WO 2001-CN243 20010226. PRIORITY: CN 2000-111967 20000310.

AB The invention provides protein and cDNA sequences for 12 kDa novel human protein cloned from fetal brain, and which have similar expression pattern

with human transglutaminase-10. The invention also relates to constructing transglutaminase gene expression vectors to prep. ***recombinant*** ***transglutaminase*** protein using prokaryote or eukaryote cells. Methods of expressing and prepg. ***recombinant*** ***transglutaminase*** protein and its antibody are described. Methods of using transglutaminase gene or protein products for the treatment of various kinds of diseases, such as cancer, blood diseases, HIV infection, immune diseases and inflammation are also disclosed.

L3 ANSWER 23 OF 57 CAPLUS COPYRIGHT 2004 ACS on STN
2001:515825 Document No. 136:165524 IgA anti-tissue transglutaminase: setting the stage for coeliac disease screening. Schuppan, Detlef; Hahn, Eckhart G. (Medical Department I, University of Erlangen-Nuernberg, Erlangen, Germany). European Journal of Gastroenterology & Hepatology, 13(6), 635-637 (English) 2001. CODEN: EJGHES. ISSN: 0954-691X. Publisher: Lippincott Williams & Wilkins.

AB A review discussed the standardization of an ELISA assay to detect IgA anti-tissue transglutaminase in coeliac disease screening. Coeliac disease is triggered in genetically predisposed individuals by the ingestion of wheat and related cereals. Affected persons raise an intestinal mucosal T-cell response against the gluten fraction of these cereals. Furthermore, they produce characteristic circulating IgA antibodies to a self-antigen present in the extracellular matrix that can be detected on tissue sections. The pos. predictive value of these endomysial, reticular or umbilical cord antibodies for coeliac disease comes close to 100%. Recently, the enzyme tissue transglutaminase was identified as the main, if not sole, endomysial autoantigen in coeliac disease. ELISA tests with tissue transglutaminase from guinea pig or the recombinant human enzyme have been established that allow a standardized and quant. detn. of IgA anti-tissue transglutaminase titers. While the published assay variants report high pos. and neg. predictive values for coeliac disease, they were applied to preselected patients from mostly single centers. Therefore, validation and in part cross-validation of a standardized assay based on guinea pig tissue transglutaminase in 38 European and non-European centers is timely. With a sensitivity of 90% and specificity of 96% relative to local diagnostic stds. the assay performed well. Considering further improvement by the use of ***recombinant*** human tissue ***transglutaminase*** as the antigen and central re-evaluation of the local stds. for confirmation of coeliac disease, this ELISA promises to become the primary tool for non-invasive diagnosis, therapy control and screening of coeliac disease. However, with an estd. prevalence of 1:100-1:200 of mostly atypical and subclin. coeliacs in Western populations, we are confronted with the question of how far mass screening is ethically feasible and cost effective.

L3 ANSWER 24 OF 57 CAPLUS COPYRIGHT 2004 ACS on STN
2001:455380 Document No. 135:104258 Polyethylene glycol enhanced refolding of the ***recombinant*** human tissue ***transglutaminase*** . Ambrus, Attila; Fesus, Laszlo (Department of Biochemistry and Molecular Biology, University of Debrecen, Debrecen, H-4012, Hung.). Preparative Biochemistry & Biotechnology, 31(1), 59-70 (English) 2001. CODEN: PBBIF4. ISSN: 1082-6068. Publisher: Marcel Dekker, Inc..

AB Tissue transglutaminase (I) forms crosslinks between Lys and Gln side-chains of polypeptide chains in a Ca2+-dependent reaction; its structural basis is still not clarified. Here, the authors demonstrate that the refolding of human recombinant I to its catalytically active form from inclusion bodies requires the presence of a helper material with higher mol. wt., but only in the initiation phase. Ca2+ and nucleotides are ascribed as effector mols. also in the early phase of structural reconstitution. Two optimal concns. of polyethylene glycol and a relatively long time scale for the evolution of the final structure were identified. The optimized refolding procedure is reported.

L3 ANSWER 25 OF 57 CAPLUS COPYRIGHT 2004 ACS on STN
2001:438108 Document No. 135:120589 Radioimmunoassay to detect antitransglutaminase autoantibodies is the most sensitive and specific screening method for celiac disease. Bonamico, M.; Tiberti, C.; Picarelli, A.; Mariani, P.; Rossi, D.; Cipolletta, E.; Greco, M.; Di Tola, M.; Sabbatella, L.; Carabba, B.; Magliocca, F. M.; Strisciuglio, P.; Di Mario, U. (Istituto di Clinica Pediatrica, Dipartimento di Scienze Cliniche, Medicina Sperimentale e Patologia, Universita "La Sapienza",

Rome, Italy). American Journal of Gastroenterology, 96(5), 1536-1540 (English) 2001. CODEN: AJGAAR. ISSN: 0002-9270. Publisher: Elsevier Science Inc..

AB The aim of this study was to establish the most sensitive and specific screening method for celiac disease. We tested three methods based on different principles, which all detect autoantibodies against the same antigen (tissue transglutaminase). Sixty-two celiac children at the first biopsy (group 1), 78 celiac children on a gluten-free diet (group 2), 14 celiac children on a gluten-challenge (group 3), and 56 controls with a normal duodenal mucosa (group 4) were studied. The methods used were: (1) radioimmunopptn. assay using ***recombinant*** tissue ***transglutaminase*** (RIA); (2) com. enzyme immunoassay using guinea pig tissue transglutaminase (ELISA); and (3) indirect immunofluorescence method for detection of antiendomysium antibodies (IF-EMA). RIA antitransglutaminase autoantibodies were detected in 100% of group 1, 43.6% of group 2, 100% of group 3, and none of the control subjects. ELISA antitransglutaminase autoantibodies were detected in 90.3% of group 1, 9% of group 2, 78.6% of group 3, and in none of the control subjects. IF-EMA were detected in 95.2% of group 1, 11.5% of group 2, 92.3% of group 3, and 1.8% of the controls. Our results demonstrate a very high sensitivity and specificity of the RIA method to detect antitransglutaminase autoantibodies in comparison to ELISA and IF-EMA assays. We can explain this finding with the use of human recombinant antigen and the increased capacity of the RIA method to detect low titers of autoantibodies. If our data are confirmed by studies on larger series, tissue transglutaminase RIA could be proposed as the best screening method for celiac patients.

L3 ANSWER 26 OF 57 CAPLUS COPYRIGHT 2004 ACS on STN

2001:247521 Document No. 134:291099 Recombinant expression and extracellular secretion of exogenous proteins in coryneform bacteria by protease cleavage of proprotein-signal peptide fusion construct. Kikuchi, Yoshimi; Date, Masayo; Umezawa, Yukiko; Yokoyama, Keiichi; Matsui, Hiroshi (Ajinomoto Co., Inc., Japan). PCT Int. Appl. WO 2001023591 A1 20010405, 151 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (Japanese). CODEN: PIXXD2. APPLICATION: WO 2000-JP6780 20000929. PRIORITY: JP 1999-280098 19990930; JP 2000-194043 20000628.

AB A process for the prodn. of a exogenous secretory protein by using a coryneform bacterium is disclosed. The method comprises making a coryneform bacterium to produce an industrially useful exogenous protein (in particular, transglutaminase) and efficiently secreting the product outside the cells (i.e., secretion). A target exogenous protein is produced by using an expression construct wherein the target exogenous protein gene sequence contg. the pro-structure part is ligated to the downstream of a sequence encoding the signal peptide originating in a coryneform bacterium, transferring this expression type gene construct into the coryneform bacterium, culturing the thus transformed coryneform bacterium, and treating the extracellularly released protein with a protease, etc. to cleave and eliminate the pro-part. Use of the signal peptide of S-layer protein (S-protein) such as Corynebacterium ammoniagenes slpA or Corynebacterium glutamicum PS1 and PS2, with a Streptomyces albogriseolus serine protease SAM-P45 and Streptomyces mobaraense proline-specific peptidase svPEP, for the prodn. of Streptoverticillium mobaraense or Streptoverticillium cinnamoneum pro-transglutaminase, is described. Streptomyces mobaraense proline-specific peptidase svPEP, active toward Ala-Ala-Pro-pNA, Ala-Phe-Pro-pNA, and Phe-Arg-Ala-Pro-pNA, and inhibited by phenylmethyl sulfonyl fluoride (PMSF) or aminoethyl benzene sulfonyl fluoride hydrochloride, is specifically used.

L3 ANSWER 27 OF 57 CAPLUS COPYRIGHT 2004 ACS on STN

2001:23423 Document No. 134:315968 Triggered release of calcium from lipid vesicles: a bioinspired strategy for rapid gelation of polysaccharide and protein hydrogels. Westhaus, E.; Messersmith, P. B. (Biomedical

Engineering Department, Northwestern University, Evanston, IL, 60208, USA). Biomaterials, 22(5), 453-462 (English) 2001. CODEN: BIMADU. ISSN: 0142-9612. Publisher: Elsevier Science Ltd..

AB The bioinspired strategy of triggered release of Ca2+ from liposomal compartments was used to induce rapid gelation of polysaccharide and protein-based hydrogels. Thermally triggerable liposomes were designed by entrapping CaCl2 within liposomes constructed of 90% dipalmitoylphosphatidylcholine and 10% dimyristoylphosphatidylcholine. These liposomes released greater than 90% of entrapped Ca2+ when heated to 37.degree.C. A precursor fluid contg. liposomes suspended in aq. sodium alginate remained fluid for several days at room temp. but gelled rapidly when heated to 37.degree.C, as a result of Ca2+ release and formation of crosslinked Ca-alginate. Alternatively, thermally triggered Ca2+ release from liposomes was used to activate enzyme-catalyzed crosslinking of proteins to form hydrogels. A mixt. of Ca-loaded liposomes, fibrinogen, and a Ca2+-dependent ***transglutaminase*** enzyme (either human ***recombinant*** FXIII or guinea pig liver transglutaminase) remained fluid indefinitely when stored at room temp., but gelled rapidly when heated to 37.degree.C. SDS-PAGE of the reaction mixt. revealed that gelation was due to enzymic crosslinking of the .alpha. and .gamma. chains of fibrinogen, and oscillating rheometry revealed gel formation within 10 min of heating to 37.degree.C. This new approach may be useful for developing rapidly gelling injectable biomaterials that can be stored at room temp. and injected in a minimally invasive manner into a body tissue or cavity, upon which rapid solidification would occur. This versatile bioinspired strategy could be utilized for the delivery of biomaterials for tissue repair and reconstruction, and local site-directed drug delivery.

L3 ANSWER 28 OF 57 CAPLUS COPYRIGHT 2004 ACS on STN

2000:787259 Document No. 134:67902 Characterization of human ***recombinant*** ***transglutaminase*** 1 purified from baculovirus-infected insect cells. Hitomi, Kiyotaka; Yamagiwa, Yoshihisa; Ikura, Koji; Yamanishi, Kiyofumi; Maki, Masatoshi (Department of Applied Molecular Biosciences, Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya, 464-8601, Japan). Bioscience, Biotechnology, and Biochemistry, 64(10), 2128-2137 (English) 2000. CODEN: BBBIEJ. ISSN: 0916-8451. Publisher: Japan Society for Bioscience, Biotechnology, and Agrochemistry.

AB Transglutaminase 1 (I) is required for the formation of a cornified envelope in stratified squamous epithelia. Recombinant human I expressed in baculovirus-infected cells was purified in a sol. form with a mol. wt. of 92 kDa. Recombinant I was susceptible to limited proteolysis by both .mu.- and m-calpains. Although the proteolysis did not induce the elevation of the specific enzyme activity of I, the requirement of Ca2+ in the enzymic reaction was reduced. Furthermore, the effects of GTP, NO, and sphingosylphosphocholine, known as regulatory factors for tissue-type isoenzyme (transglutaminase 2), on the enzymic activity of I were investigated.

L3 ANSWER 29 OF 57 CAPLUS COPYRIGHT 2004 ACS on STN

2000:384869 Document No. 133:280779 Human ***recombinant*** tissue ***transglutaminase*** ELISA: an innovative diagnostic assay for celiac disease. Sblattero, D.; Berti, I.; Trevisiol, C.; Marzari, R.; Tommasini, A.; Bradbury, A.; Fasano, A.; Ventura, A.; Not, T. (Department of Pediatrics, IRCCS "Burlo Garofolo", Trieste, Italy). American Journal of Gastroenterology, 95(5), 1253-1257 (English) 2000. CODEN: AJGAAR. ISSN: 0002-9270. Publisher: Elsevier Science Inc..

AB OBJECTIVE: Tissue transglutaminase is the autoantigen recognized by the sera of celiac patients. An ELISA based on guinea-pig tissue transglutaminase was recently used to measure serum tissue transglutaminase antibodies for the diagnosis of celiac disease. We det. the sensitivity and specificity of an ELISA test based on the use of human ***recombinant*** ***transglutaminase*** , compared with the guinea pig transglutaminase ELISA and IgA antiendomysium antibodies. METHODS: Serum samples were tested from 65 patients with intestinal biopsy proven celiac disease, from 10 patients with Crohn's disease, and from 150 healthy blood donors. RESULTS: Human transglutaminase ELISA identified 64 of 65 celiac patients, whereas the guinea pig transglutaminase ELISA and IgA antiendomysium antibodies identified 58 of 65 and 60 of 65 subjects, resp. The three tests showed comparable specificity. CONCLUSIONS: These

results proved that the human tissue transglutaminase-based ELISA represents a cost-effective strategy for identifying both symptomatic and atypical forms of celiac disease and could mean that intestinal biopsy need no longer be the gold std. for diagnosing this clin. condition. Furthermore, early identification and treatment of patients with celiac disease in an outpatient setting could have significant implications for reducing long-term morbidity and can produce major savings in future health care costs.

L3 ANSWER 30 OF 57 CAPLUS COPYRIGHT 2004 ACS on STN
2000:324962 Document No. 133:217531 Recombinant factor XIII improves established experimental colitis in rats. D'Argenio, Giuseppe; Grossman, Angelika; Cosenza, Vittorio; Della Valle, Nicola; Mazzacca, Gabriele; Bishop, Paul D. (Gastrointestinal Unit, School of Medicine, Federico II University, Naples, 80131, Italy). Digestive Diseases and Sciences, 45(5), 987-997 (English) 2000. CODEN: DDSCDJ. ISSN: 0163-2116. Publisher: Kluwer Academic/Plenum Publishers.
AB Factor XIII (FXIII) is the plasma-borne transglutaminase involved in fibrin clot stabilization and wound healing. FXIII levels in the plasma of patients with inflammatory bowel diseases are lower than normal and there is a significant inverse correlation of FXIII levels with clin. severity. Moreover, uncontrolled studies report beneficial effects of FXIII supplementation in patients resistant to conventional therapies. We investigated the effects of i.v. recombinant FXIII (rFXIII) treatment in exptl. induced rat colitis to verify that FXIII was the active agent in plasma FXIII concs. and to better understand the potential therapeutic use of this protein. Colitis was induced by instillation of 12% 2,4,6-trinitrobenzenesulfonic acid (TNBS) in 50% ethanol into the colon of male Wistar rats. Rats were treated with 0.65 mg/kg rFXIII or vehicle (i.v.) daily for 10 days. Treatment was started either immediately after TNBS/EtOH instillation (to evaluate effects on developing lesions) or seven days later (to evaluate effects on established lesions). In both cases rats were killed either immediately after the end of treatment (to evaluate immediate effects) or 17 days later (to evaluate long-lasting effects). The effects of rFXIII were compared to pos. (5-amino-2-hydroxybenzoic acid) control over a 35-day time course. The severity of lesions was detd. by colon wt. and macroscopic and histol. scores. Transglutaminase activity was measured in both colon tissue and serum. RFXIII treatment reduced lesion severity significantly not only in developing but also in established lesions. Improvements in healing persisted at least 18 days after treatment was discontinued. Serum and tissue transglutaminase levels were restored by rFXIII treatment. In conclusion, pure rFXIII is as effective as plasma FXIII concs. in a rat model of exptl. colitis. In addn., rFXIII significantly improves the healing of preexisting lesions, a characteristic useful in treatment of human inflammatory bowel diseases.

L3 ANSWER 31 OF 57 CAPLUS COPYRIGHT 2004 ACS on STN
2000:314820 Document No. 132:333383 Glycosylated proteins having reduced allergenicity. Olsen, Arne Agerlin; Roggen, Erwin Ludo; Ernst, Steffen (Novo Nordisk A/S, Den.). PCT Int. Appl. WO 2000026354 A1 20000511, 74 pp. DESIGNATED STATES: W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1999-DK540 19991012. PRIORITY: DK 1998-1401 19981030; DK 1998-1551 19981125; DK 1999-682 19990517; DK 1999-1419 19991004.
AB Provided is a method of producing a glycosylated protein variant having reduced allergenicity in animals, including man, as compared to parent protein. The method comprises constructing a DNA mol. encoding the protein variant having at least on sub-sequence encoding an addnl. glycosylation site, introducing the DNA mol. into a suitable host capable of glycosylation, and recovering glycosylated protein variant from the culture medium. The recombinant proteins having addnl. glycosylation site are identified by screening of phage display library. The protein is an industrially applicable enzyme such as protease, lipase, phytase, polysaccharide lyase, oxidoreductase, transglutaminase, glycosyl hydrolase

and glucose isomerase. The glycosylated enzyme variant is useful in medical product, pharmaceutical, detergent or personal care product, food and feed (baking) industry, and textile industry.

L3 ANSWER 32 OF 57 CAPLUS COPYRIGHT 2004 ACS on STN
2000:90506 Document No. 132:261815 Transglutaminase-catalyzed formation of Alzheimer-like insoluble complexes from recombinant tau. Balin, Brian J.; Appelt, Denah M. (Division of Neuroscience, Departments of Pathology and Microbiology/Immunology, Philadelphia College of Osteopathic Medicine, Philadelphia, PA, USA). Methods in Molecular Medicine, 32(Alzheimer's Disease: Methods and Protocols), 395-403 (English) 2000. CODEN: MMMEFN. Publisher: Humana Press Inc..
AB Alzheimer's disease is a progressive neurodegenerative disease in which abnormal filamentous inclusions accumulate in dystrophic and dying nerve cells. These inclusions have been described as neurofibrillary tangles of which paired helical filaments are primary constituents. The paired helical filaments are composed of a microtubule assocd. protein tau which has undergone posttranslational modifications such as phosphorylation, glycation, and crosslinking by transglutaminase. In the paper the following protocols used for studying tau complexes formation are described: ***recombinant*** tau purifn., ***transglutaminase*** incubation, SDS-PAGE and western blotting, and immune electron microscopy.

L3 ANSWER 33 OF 57 CAPLUS COPYRIGHT 2004 ACS on STN
1999:799591 Document No. 132:133922 ***Recombinant*** human tissue ***transglutaminase*** ELISA for the diagnosis of gluten-sensitive enteropathy. Sardy, Miklos; Odenthal, Uwe; Karpati, Sarolta; Paulsson, Mats; Smyth, Neil (Department of Dermato-Venereology, Semmelweis University of Medicine, Budapest, H-1085, Hung.). Clinical Chemistry (Washington, D. C.), 45(12), 2142-2149 (English) 1999. CODEN: CLCHAU. ISSN: 0009-9147. Publisher: American Association for Clinical Chemistry.
AB Background: Tissue transglutaminase (TGc) has recently been identified as the major, if not the sole, autoantigen of gluten-sensitive enteropathy (GSE). We developed and validated an ELISA based on the human recombinant antigen and compared it to existing serol. tests for GSE [guinea pig TGc ELISA and endomysium antibody (EMA) test]. Methods: Human TGc was expressed in the human embryonic kidney cell line 293-EBNA as a C-terminal fusion protein with the eight-amino acid Strep-tag II allowing one-step purifn. via streptavidin affinity chromatog. We carried out ELISA assays for IgA antibodies against TGc using calcium-activated human and guinea pig TGc. The sera were also tested on monkey esophagus sections by indirect immunofluorescence for IgA EMA. We examd. 71 serum samples from patients with GSE (38 with celiac disease, 33 with dermatitis herpetiformis), including 16 on therapy, and 53 controls. Results: The human TGc could be expressed and purified as an active enzyme giving a single band on a Coomassie-stained gel. The mean intra- and interassay CVs for the human TGc ELISA were 3.2% and 9.2%, resp. The area under the ROC curve was 0.999. The specificity and sensitivity were 98.1% (95% confidence interval, 95.7-100%) and 98.2% (95.9-100%), resp. Conclusions: The human TGc ELISA was somewhat superior to the guinea pig TGc ELISA, and was as specific and sensitive as the EMA test. The human TGc-based ELISA is the method of choice for easy and noninvasive screening and diagnosis of GSE.

L3 ANSWER 34 OF 57 CAPLUS COPYRIGHT 2004 ACS on STN
1999:659654 Document No. 131:283323 Bacterial ***transglutaminases*** and genes and ***recombinant*** pro- ***transglutaminase*** for use in food, cosmetic and pharmaceutical industries. Fuchsbauer, Hans-Lothar; Pasternack, Ralf; Dorsch, Simone; Otterbach, Jens; Robenek, Isabella; Mainusch, Martina; Dauscher, Christine (Germany). Ger. Offen. DE 19814860 A1 19991007, 44 pp. (German). CODEN: GWXXBX. APPLICATION: DE 1998-19814860 19980402.
AB The sequences of the pro domains and the mature transglutaminases of Streptoverticillium mobaraense and S. fervens melrosporus and the corresponding nucleic acids encoding these peptides/proteins are disclosed. Vectors expressing prepro-, pro- and mature transglutaminases of S. mobaraense and S. fervens melrosporus, recombinant bacteria transformed with these vectors, and a method for prepg. pro- ***transglutaminase*** with such ***recombinant*** bacteria are further disclosed. The transglutaminases may be used to crosslink proteins and as such may be used in the food, cosmetic and pharmaceutical

industries.

L3 ANSWER 35 OF 57 CAPLUS COPYRIGHT 2004 ACS on STN
1999:548289 Document No. 131:283195 Characterization of ***recombinant*** mouse epidermal-type ***transglutaminase*** (TGase 3): regulation of its activity by proteolysis and guanine nucleotides. Hitomi, Kiyotaka; Kanehiro, Shinya; Ikura, Koji; Maki, Masatoshi (Department of Applied Molecular Biosciences, Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya, 464-8601, Japan). Journal of Biochemistry (Tokyo), 125(6), 1048-1054 (English) 1999. CODEN: JOBIAO. ISSN: 0021-924X. Publisher: Japanese Biochemical Society.
AB Epidermal-type TGase (TGase 3) is involved in the formation of the cornified cell envelope by crosslinking a variety of structural proteins in the epidermis. Unknown proteases activate this enzyme from the zymogen form by limited proteolysis during epidermal differentiation. It has been difficult to isolate sufficient quantities of native enzymes from tissues for biochem. studies of the properties of TGase 3. In this paper, we circumvented these problems by expressing recombinant full-length mouse TGase 3 in a baculovirus system, and purifying it to homogeneity by successive chromatog. and HPLC. Treatment of the purified recombinant protein with dispase, a bacterial protease known to activate zymogens, produced activated TGase 3. The migration of TGase 3 zymogen in SDS-polyacrylamide gel electrophoresis was anomalous when the proTGase 3 was pre-incubated with calcium ion. GTP inhibited the enzymic activity of recombinant TGase 3. Calpain, a calcium-dependent neutral protease, was a candidate protease, but had no effect on the activation of TGase 3 zymogen.

L3 ANSWER 36 OF 57 CAPLUS COPYRIGHT 2004 ACS on STN
1999:432803 Document No. 131:86572 Antibodies to human ***recombinant*** tissue ***transglutaminase*** measured by radioligand assay. Evidence for high diagnostic sensitivity for celiac disease. Seissler, J.; Boms, S.; Wohlrab, U.; Morgenthaler, N. G.; Mothes, T.; Boehm, B. O.; Scherbaum, W. A. (Diabetes Research Institute, Univ. Dusseldorf, Dusseldorf, D-40225, Germany). Hormone and Metabolic Research, 31(6), 375-379 (English) 1999. CODEN: HMMRA2. ISSN: 0018-5043. Publisher: Georg Thieme Verlag.
AB Celiac disease is assocd. with endomysial antibodies (EmA), which have recently been reported to be directed to tissue transglutaminase (tTG). To demonstrate binding of antibodies to recombinant tTG, human tTG was cloned, expressed by in vitro transcription/translation and used to develop novel radioligand assays for combined and single detection of IgA (IgA) and G (IgG)-specific antibodies. IgA and IgG-tTGA were found in 43 (95.6%) of 45 patients with newly-diagnosed celiac disease verified by biopsy. In addn., all 30 sera from patients with gastrointestinal symptoms and pos. EmA were pos. for IgA-tTGA, and all but one serum (96.7%) had antibodies of the IgG class. Receiver-operating characteristic anal. including 574 sera from healthy controls revealed a specificity of 99.5%. By means of these new assays, we identified all patients with endomysial antibodies and achieved, at equal specificity, an even improved sensitivity (95.6%) as compared to EmA (91.1%) detected by the std. immunofluorescence test. Here, we have provided direct evidence that recombinant tTG is a major target of antibodies in celiac disease. Our data suggest that tTGA measured by radioligand assay have the power to overcome the limitations of the EmA-test. This new strategy may considerably facilitate large-scale screening for silent and latent celiac disease.

L3 ANSWER 37 OF 57 CAPLUS COPYRIGHT 2004 ACS on STN
1998:657603 Document No. 130:1678 GTP-dependent conformational changes associated with the functional switch between G.alpha. and crosslinking activities in brain-derived tissue transglutaminase. Monsonego, Alon; Friedmann, Igor; Shani, Yael; Eisenstein, Miriam; Schwartz, Michal (Department of Neurobiology, The Weizmann Institute of Science, Rehovot, 76100, Israel). Journal of Molecular Biology, 282(4), 713-720 (English) 1998. CODEN: JMOBAK. ISSN: 0022-2836. Publisher: Academic Press.
AB GTP and Ca2+, two well-known modulators of intracellular signaling pathways, control a structural/functional switch between two vital and mutually exclusive activities, crosslinking and G.alpha. activity, in the same enzyme. The enzyme, a brain-derived tissue-type transglutaminase (TGase), was recently cloned by us in two forms, one of which (s-TGN) lacks a C-terminal region that is present in the other (1-TGN).

Immunoreaction with antibodies directed against a peptide present in the C-terminus of 1-TGN but missing in s-TGN suggested that this site, which is located in the C-terminal fourth domain, undergoes conformational changes as a result of interaction between 1-TGN and GTP. Site-directed mutagenesis suggested that the third domain is involved in mediating the inhibition of the crosslinking activity. These results were supported by mol. modeling, which further suggested that domains III and IV both participate in conformational changes leading to the functional switch between the Ca2+-dependent crosslinking activity and the G.alpha. activity. (c) 1998 Academic Press.

L3 ANSWER 38 OF 57 CAPLUS COPYRIGHT 2004 ACS on STN
1998:454111 Document No. 129:186118 Overproduction of DnaJ in Escherichia coli improves in vivo solubility of the ***recombinant*** fish-derived ***transglutaminase*** . Yokoyama, Kei-Ichi; Kikuchi, Yoshimi; Yasueda, Hisashi (Food Research and Development Laboratories, Ajinomoto Co. Inc., Kanagawa, 210-0801, Japan). Bioscience, Biotechnology, and Biochemistry, 62(6), 1205-1210 (English) 1998. CODEN: BBBIEJ. ISSN: 0916-8451. Publisher: Japan Society for Bioscience, Biotechnology, and Agrochemistry.
AB The overexpression of red sea bream (Pagrus major) transglutaminase (TGase, E.C. 2.3.2.13) in Escherichia coli mostly leads to the accumulation of biol. inactive enzyme. Although the soly. of the gene products could be improved by cultivation at a lower temp. (26-28.degree.), most of the synthesized TGase was still in the form of insol. aggregates. The effects of overprodn. of mol. chaperones on the intracellular soly. of newly produced recombinant TGase were examd. The over-expression of dnaK or groES/EL did not improve soly. However, DnaJ greatly increased the soly. of the recombinant TGase, resulting in active enzyme in the presence of calcium ions. Co-expression of dnaK along with dnaJ further increased the content of sol. TGase. Under our exptl. conditions, supplementation with both DnaJ and DnaK elevated the TGase activity in the producer cells by roughly 4-fold, compared with the control strain cultured at 30.degree.. Thus, the authors found that DnaJ is important in controlling the soly. of protein overproduced in E. coli.

L3 ANSWER 39 OF 57 CAPLUS COPYRIGHT 2004 ACS on STN
1998:79052 Document No. 128:240913 Identification of transglutaminase-reactive residues in S100A11. Robinson, Nancy A.; Eckert, Richard L. (Departments of Physiology and Biophysics, Case Western Reserve University School of Medicine, Cleveland, OH, 44106-4970, USA). Journal of Biological Chemistry, 273(5), 2721-2728 (English) 1998. CODEN: JBCHA3. ISSN: 0021-9258. Publisher: American Society for Biochemistry and Molecular Biology.
AB The recent finding that S100A11 is a component of the keratinocyte cornified envelope (CE) (Robinson, N. A., Lapic, S., Welter, J. F., and Eckert, R. L. (1997) J. Biol. Chem. 272, 12035-12046) suggests that S100A11 is a transglutaminase (TG) substrate. In the present study we show that S100A11 forms multimers when cultured keratinocytes are challenged by increased levels of intracellular calcium and that multimer formation is inhibited by the TG inhibitor, cystamine. These S100A11 multimers appear to be incorporated into the CE, as immunoreactive S100A11 is detected in purified envelopes prepd. from cultured cells and from foreskin epidermis. To study S100A11 as a ***transglutaminase*** substrate, ***recombinant*** human S100A11 (rhS100A11) was used in a cell-free crosslinking system. [14C]Putrescine, a primary amine, labels rhS100A11 in a TG-dependent manner. Trypsin digestion of [14C]putrescine-labeled rhS100A11 releases one radiolabeled peptide, Ala98-Lys103. The glutamine residue in this segment, Gln102, is the site of radiolabel incorporation indicating that Gln102 functions as an amine acceptor. The ability of S100A11 to form multimers indicates that it also has a reactive lysine residue that functions as an amine donor. To identify the reactive residue, we compared the high pressure liq. chromatog. profile of trypsin-digested rhS100A11 monomer to that of cross-linked rhS100A11. A unique cross-linked peptide was purified and identified as Met-Ala-Lys3-Ile-Ser-Ser-Pro-Thr-Glu-Thr-Glu-Arg cross-linked via an Lys3-Gln102 isopeptide bond to Ala-Val-Pro-Ser-Gln102-Lys. These studies show that S100A11 is post-translationally modified by transglutaminase, that it can be cross-linked to form multimers, that it is present in CEs from cultured keratinocytes and in vivo epidermis, and that Lys3 and Gln102 are specific sites of cross-link formation.

L3 ANSWER 40 OF 57 CAPLUS COPYRIGHT 2004 ACS on STN
1997:663228 Document No. 127:328356 Analysis of factor XIII substrate specificity using recombinant human factor XIII and tissue transglutaminase chimeras. Hettasch, Joann M.; Peoples, Keith A.; Greenberg, Charles S. (Department of Medicine, Duke University Medical Center, Durham, NC, 27710, USA). Journal of Biological Chemistry, 272(40), 25149-25156 (English) 1997. CODEN: JBCHA3. ISSN: 0021-9258. Publisher: American Society for Biochemistry and Molecular Biology.

AB Human factor XIII (FXIII) and tissue transglutaminase (tTG) are homologous proteins. FXIII requires thrombin for activation and cross-links the .gamma. chains of fibrinogen more efficiently than the A.alpha. chains. TTG is thrombin-independent and forms predominantly A.alpha. and A.alpha.-.gamma. chain complexes. Previous work from this lab. demonstrated that amino acid residues within exon 7 of FXIII were important for catalysis (Hettasch, J. M., and Greenberg, C. S. (1994) J. Biol. Chem. 269, 28309-28313). To det. to what extent the primary amino acid sequence within exon 7 defines substrate specificity, exon 7 of FXIII was replaced with the corresponding exon of tTG using gene splicing by overlap extension. Other work from this lab. (Achyuthan, K. E., Slaughter, T. F., Santiago, M. A., Enghild, J. J., and Greenberg, C. S. (1993) J. Biol. Chem. 268, 21284-21292) using synthetic peptides identified two other domains that might play a role in substrate recognition (located in exons 3 and 5). Therefore, recombinant chimeras of FXIII/tTG were also created in which these two exons were exchanged. FXIII, tTG, and chimeras 3, 5, and 7 were expressed in Escherichia coli, purified, and the nature of the fibrin crosslinking pattern of these five proteins was detd. by immunoblot anal. FXIII preferentially formed the .gamma.-.gamma. dimer, whereas tTG formed A.alpha.-.gamma. complexes. Chimera 7 formed A.alpha.-.gamma. complexes that resembled the crosslinking pattern of tTG. This finding demonstrates that the primary amino acid sequence of exon 7 of tTG confers some of the specificity for the A.alpha. and A.alpha.-.gamma. cross-link pattern characteristic of tTG. Chimera 5 exhibited reduced crosslinking activity (50% of FXIII activity) but still retained preference for formation of the .gamma.-.gamma. dimer, whereas chimera 3 was not active. In conclusion, exchanging the primary amino acid sequence of the active site exon of human FXIII with that of human tTG modifies the enzyme such that the fibrin crosslinking pattern more closely resembles that of tTG (A.alpha. and A.alpha.-.gamma. complexes) instead of FXIII (.gamma.-.gamma. dimers).

L3 ANSWER 41 OF 57 CAPLUS COPYRIGHT 2004 ACS on STN
1997:16869 Document No. 126:102504 The association of tissue ***transglutaminase*** with human ***recombinant*** tau results in the formation of insoluble filamentous structures. Appelt, Denah M.; Balin, Brian J. (Department of Anatomy and Neurobiology, Allegheny University of the Health Sciences, Broad and Vine Streets, NCB RM 5602, MS #435, Philadelphia, PA, 19102-1192, USA). Brain Research, 745(1,2), 21-31 (English) 1997. CODEN: BRREAP. ISSN: 0006-8993. Publisher: Elsevier.

AB To det. possible mechanisms by which NFTs (neurofibrillary tangles) are formed in Alzheimer's disease (AD), the authors investigated the ability of tissue transglutaminase (TGase) to convert human recombinant tau proteins into insol. filamentous structures. TGase derived from guinea pig liver was activated by calcium to catalyze the in vitro crosslinking of the largest sol. recombinant tau isoform (htau40) into insol. complexes as detd. by electrophoresis following incubation in 4 M urea and SDS. The TGase-catalyzed formation of these insol. complexes occurred within 15 min to 24 h and the decreased migration of the insol. material correlated with increased calcium concns. ranging from 2 mM to 50 mM when analyzed electrophoretically. TGase-treated human recombinant tau formed filamentous structures in vitro that were immunoreactive with antibodies to tau and TGase. These structures retained the insol. characteristics typical of AD PHF (paired helical filaments)/NFTs. Immunolabeling with the TGase antibody revealed that TGase is assocd. with the filaments formed from human recombinant tau in vitro as well as with PHFs isolated from NFTs from AD brains. These novel findings support an in vitro model for investigating the biophys. changes that occur in converting sol. tau proteins into an insol. matrix consistent with the insol. PHFs/NFTs which may contribute to neuronal degeneration and cell death in the AD brain.

L3 ANSWER 42 OF 57 CAPLUS COPYRIGHT 2004 ACS on STN
1996:577682 Document No. 125:214268 Bacillus-derived

transglutaminase gene sequence, ***recombinant*** enzyme production, and use in food industry for crosslinked protein production. Kobayashi, Katsunori; Yamanaka, Shigeru; Miwa, Kiyoshi; Suzuki, Shunichi; Eto, Yuzuru; Tanita, Yuko; Yokozeki, Kenzo; Hashiguchi, Kenichi (Ajinomoto Co., Inc., Japan). Eur. Pat. Appl. EP 726317 A2 19960814, 39 pp. DESIGNATED STATES: R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, MC, NL, PT, SE. (English). CODEN: EPXXDW. APPLICATION: EP 1996-101905 19960209. PRIORITY: JP 1995-21963 19950209; JP 1995-226316 19950904; JP 1996-13072 19960129.

AB The invention relates to a Ca2+-independent transglutaminase derivable from cells of the genus Bacillus having the following properties: a) The suitable pH-value for it si from about 7 to about 9, b) the suitable temp. for it is from about 40.degree.C to about 65.degree.C, c) it has an activity of 50% or more in the presence of 5 mM Ca2+, and d) it has a mol. wt. of from about 28,000 to about 30,000 (as measured by SDS/PAGE). The transglutaminase is obtainable form spores of Bacilli. The present invention further relates to vectors and microorganisms contg. a DNA fragment encoding said transglutaminase and to methods for producing the ***recombinant*** ***transglutaminase*** .

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1996:537686 Document No. 125:189371 Transglutaminases from Oomycetes, their production with recombinant cells, and their use in foods and cosmetics. Bech, Lisbeth; Rasmussen, Grethe; Halkier, Torben; Okada, Mariko; Andersen, Lene Nonboe; Kauppinen, Markus Sakari; Sandal, Thomas (Novo Nordisk A/s, Den.). PCT Int. Appl. WO 9622366 A1 19960725, 75 pp. DESIGNATED STATES: W: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE. (English). CODEN: PIXXD2. APPLICATION: WO 1996-DK31 19960119. PRIORITY: DK 1995-61 19950119.

AB Transglutaminase and transglutaminase prepns. can be produced by lower fungi belonging to the class Oomycetes and unprecedented high-level expression is achievable by growing these coenocytium forming organisms, esp. Pythium sp. and Phytophthora sp.; and a ***recombinant*** ***transglutaminase*** has been cloned and expressed, the enzyme and enzyme prepns. being useful for crosslinking proteins, e.g. in flour, baked products, meat products, fish products, cosmetics, cheese, milk products, gelled food products and leather finishing, or as a glutaminase, e.g. in bread and other baked gluten-contg. food products. Oomycetes producing transglutaminase were identified and some transglutaminases were characterized. Phytophthora cactorum transglutaminase cDNA was expressed in Aspergillus. The strengthening of gluten in wheat flour dough by transglutaminase was demonstrated.

L3 ANSWER 44 OF 57 CAPLUS COPYRIGHT 2004 ACS on STN

1996:333024 Document No. 125:27680 Human prostate or placental ***transglutaminase*** cDNA sequences, ***recombinant*** enzyme production, and uses of transglutaminases. O'hara, Patrick J.; Grant, Francis J.; Sheppard, Paul O. (Zymogenetics, Inc., USA). U.S. US 5514579 A 19960507, 19 pp., Cont.-in-part of U.S. Ser. No. 816, 284, abandoned. (English). CODEN: USXXAM. APPLICATION: US 1992-998973 19921230. PRIORITY: US 1991-816284 19911231.

AB Human prostatic and placental transglutaminases are identified and cloned. The human transglutaminases herein are useful for, inter alia, therapeutic wound repair, closure of skin grafts, stabilizing food prepns., and markers for identifying agents which act as agonists or antagonists of cellular apoptosis.

L3 ANSWER 45 OF 57 CAPLUS COPYRIGHT 2004 ACS on STN

1996:332815 Document No. 125:4415 Microbial transglutaminases, their production, gene cloning and sequence, and use for protein crosslinking. Bech, Lisbeth; Noerrevang, Iben Angelica; Halkier, Torben; Rasmussen, Grethe; Schaefer, Thomas; Andersen, Jens Toenne (Novo Nordisk A/s, Den.). PCT Int. Appl. WO 9606931 A1 19960307, 95 pp. DESIGNATED STATES: W: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO

1995-DK347 19950828. PRIORITY: DK 1994-990 19940826; DK 1995-947 19950824.

AB Transglutaminase prepns. are producible by a wide range of fungi, esp. ascomycotina, basidiomycotina and zygomycota, and gram-neg. and gram-pos. bacteria, esp. Streptomyces lydicus, NRRL B-3446. Several bacterial and fungal transglutaminases were characterized with regard to temp., pH, and Ca2+ dependencies. The transglutaminases from S. lydicus and S. platensis were purified and structurally characterized by peptide sequencing; fermentative prodn. of S. lydicus transglutaminase yielded .apprx.180 mg/L undiluted broth, much higher than prior art yields of 2.47 units/mL. A DNA construct encoding the novel transglutaminase from S. lydicus and comprising the DNA sequence obtainable from the plasmid in E. Coli, DSM 10175, is also described together with a method of producing the transglutaminases, a compn. comprising the transglutaminase, and a method for producing a gel or protein gelation compn. The transglutaminase prepn. can be used for protein crosslinking in flour, meat products, fish products, cosmetics, cheese, milk products, gelled food products, and shoe shine.

L3 ANSWER 46 OF 57 CAPLUS COPYRIGHT 2004 ACS on STN

1996:51813 Document No. 124:110585 GTP hydrolysis by human tissue transglutaminase homolog. Fraij, Bassam M. (Department Biochemistry Molecular Biology, Oklahoma State University, Stillwater, OK, 74078-3035, USA). Biochemical and Biophysical Research Communications, 218(1), 45-9 (English) 1996. CODEN: BBRCA9. ISSN: 0006-291X. Publisher: Academic.

AB Human tissue transglutaminase homolog cDNA was expressed in E. coli to analyze the catalytic characteristics. The transglutaminase homolog was purified by immunoaffinity chromatog. Specificity of GTP binding by the homolog was demonstrated by photoaffinity labeling in the absence or presence of GTP-.gamma.-S. The homolog had GTPase activity with an apparent Km value of 1.8 .mu.M, several-fold lower than the reported Km values for the native tissue transglutaminase. GTPase activity was inhibited by guanine nucleotides in order GTP-.gamma.-S > GDP > GMP. The higher GTPase activity of the homolog may be related to the signaling events function.

L3 ANSWER 47 OF 57 CAPLUS COPYRIGHT 2004 ACS on STN

1995:825253 Document No. 124:2054 Cloning, characterization, and tissue distribution of porcine SPAI, a protein with a transglutaminase substrate domain and the WAP motif. Kuroki, Jun; Hosoya, Tomoko; Itakura, Makoto; Hirose, Shigehisa; Tamechika, Ichiro; Yoshimoto, Takanobu; Ghoneim, Magdy A.; Nara, Kiyomitsu; Kato, Akira; et al. (Tsukuba Res. Lab., Eisai Co., Ltd., Tsukuba, 300-26, Japan). Journal of Biological Chemistry, 270(38), 22428-33 (English) 1995. CODEN: JBCHA3. ISSN: 0021-9258. Publisher: American Society for Biochemistry and Molecular Bio logy.

AB The primary and gene structures and tissue distribution of porcine SPAI-2, a protein that belongs to the WAP protein superfamily and has a sodium-potassium ATPase inhibitory activity, were detd. by mol. cloning and Northern anal. A full-length cDNA clone was isolated from a porcine duodenum cDNA library. The cDNA insert encoded a polypeptide of 187 amino acids, which is composed of 3 domains: a hydrophobic presequence of 21 amino acids, a prosegment of 105 amino acids ending with Asp126, and the mature SPAI-2 sequence of 61 amino acids beginning with Pro127. The prosegment contained 16 repeats of a hexapeptide that is highly homologous to the repetitive sequence found in the transglutaminase domain of the human elafin, an elastase-specific inhibitor that also belongs to the WAP superfamily. The repetitive sequence was demonstrated to be a good substrate of ***transglutaminase*** using a ***recombinant*** prepn. produced in Escherichia coli. A porcine genomic library was then screened for the SPAI gene. Characterization and sequencing of pos. clones indicated that the gene is similar to the elafin gene, having 3 exons encoding the 5'-untranslated region and signal sequence, pro-SPAI, and 3'-untranslated region, resp. Northern blot anal. revealed intestine-specific expression of SPAI mRNA; the message was esp. abundant in the small intestine. ProSPAI was also found in the circulation. The similarity of proSPAI to elafin in the domain structure, the acid-labile nature of the cleavage site (Asp126-Pro127), and the fact that the major form of SPAI in the plasma is proSPAI strongly suggest that proSPAI is not the precursor but rather it is the native form of SPAI. Like elafin, therefore, SPAI appears to be a new type of biol. active substance with a transglutaminase substrate domain that acts as an anchoring sequence.

L3 ANSWER 48 OF 57 CAPLUS COPYRIGHT 2004 ACS on STN
1995:530288 Document No. 123:28551 Site-directed mutation in conserved anionic regions of guinea pig liver transglutaminase. Ikura, Koji; Yu, Chi; Nagao, Masaya; Sasaki, Ryuzo; Furuyoshi, Setsuo; Kawabata, Nariyoshi (Dep. of Chemistry and Materials Technology, Kyoto Inst. of Technology, Kyoto, 606, Japan). Archives of Biochemistry and Biophysics, 318(2), 307-13 (English) 1995. CODEN: ABBIA4. ISSN: 0003-9861. Publisher: Academic.

AB Transglutaminases (EC 2.3.2.13) catalyze the formation of .epsilon.-(.gamma.-glutamyl)lysine cross-links and the substitution of primary amines for the .gamma.-carboxamide groups of protein-bound glutamine residues. There are conserved anionic regions in transglutaminases, some of which are thought to be possible calcium-binding sites. By site-directed mutagenesis, three mutant forms of ***recombinant*** guinea-pig liver ***transglutaminase*** , in which some acidic amino acid residues in two conserved regions became nonionic, were expressed in Escherichia coli: TGM1, with Asp-231 and -232 changed to Asn; TGM2, with Glu-445, -448, -449, -450, and -452 changed to Gln; and TGM3, with the mutations of both TGM1 and TGM2. The size and level of synthesis of the mutant proteins were unchanged when monitored by immunoblotting. All mutants retained enzyme activity, and their apparent Km values for substrates during histamine incorporation into acetyl .alpha.s1-casein were similar to those of the wild-type enzyme, but their Vmax values were smaller. The deamidation rate of glutamine residues in the acetyl .alpha.s1-casein was unaffected, but the rate of protein crosslinking catalyzed by these mutants was very low. All mutations caused with the enzyme a decrease in the sensitivity to activation by calcium and an increase in the sensitivity to inhibition by GTP. These results indicated that the neg. charges of some acidic amino acid residues in the two conserved anionic regions of transglutaminase are not essential for its activity but the loss of their neg. charges affects some catalytic properties.

L3 ANSWER 49 OF 57 CAPLUS COPYRIGHT 2004 ACS on STN
1995:434127 Document No. 122:259296 Immunochemical analyses of human plasma fibronectin-cytosolic transglutaminase interactions. Achyuthan, Komandoor E.; Goodell, R. Jeff; Kennedye, James R.; Lee, Kyung N.; Henley, Anna; Stiefer, John R.; Birckbichler, Paul J. (Noble Center For Biomedical Research, Oklahoma Medical Research Foundation, 825 NE 13th Street, Oklahoma City, USA). Journal of Immunological Methods, 180(1), 69-79 (English) 1995. CODEN: JIMMBG. ISSN: 0022-1759. Publisher: Elsevier.

AB Fibronectin is a glycoprotein involved in cell adhesion, tissue organization and wound healing. Transglutaminase binding and covalent crosslinking of fibronectin are physiol. important reactions. Here, microtiter plate-based immunochem. methods to analyze cytosolic transglutaminase-human plasma fibronectin interactions are described. The immunochem. method was sensitive, specific, species-independent, and capable of simultaneously analyzing 96 samples for binding. Binding was time-, temp.-, and concn.-dependent and demonstrable with either protein immobilized to the plastic. The assay detected 1-5 ng transglutaminase or 50 pg fibronectin and was comparable in sensitivity to ELISAs. CaCl2 (8 mM) enhanced transglutaminase binding by 2-fold. Molar concns. of NaCl or millimolar concns. of chloride salts of Ba, Cu, or Zn inhibited binding by 50-60%. The binding was also competitively blocked by sol. fibronectin (IC50 = 2.3 nM) or by anti-fibronectin IgG (IC50 = 0.5 .mu.M). Inclusion of dithiothreitol or 2-mercaptoethanol during binding resulted in a concn.-dependent inhibition of transglutaminase-fibronectin interactions (IC50 = 1.5 mM and 20 mM, resp.). A complex of anti-transglutaminase IgG-transglutaminase-fibronectin-anti-fibronectin IgG suggested that the binding sites and antibody epitopes could overlap, but are distinct and surface-exposed in the 2 proteins. Liver transglutaminase bound fibronectin 30-50% less compared to erythrocyte transglutaminase. Fibronectin-transglutaminase affinity was adequate for quantitating either antigen in lysates of lung fibroblasts, breast carcinomas, or Escherichia coli. These immunochem. analyses will be useful for detg. the affinity and mapping the domains involved in antibody recognition or protein-protein interactions using ***recombinant*** mols. of ***transglutaminase*** and fibronectin.

L3 ANSWER 50 OF 57 CAPLUS COPYRIGHT 2004 ACS on STN

1994:26368 Document No. 120:26368 Site-directed mutagenesis of human tissue transglutaminase: Cys-277 is essential for transglutaminase activity but not for GTPase activity. Lee, Kyung N.; Arnold, Shelly A.; Birckbichler, Paul J.; Patterson, Manford K., Jr.; Fraij, Bassam M.; Takeuchi, Yutaka; Carter, Henry A. (The Samuel Roberts Noble Foundation, Inc., Biomedical Division, Ardmore, OK, USA). Biochimica et Biophysica Acta, 1202(1), 1-6 (English) 1993. CODEN: BBACAQ. ISSN: 0006-3002.

AB Transglutaminases (TGases; EC 2.3.2.13) catalyze an acyl-transfer reaction between peptide-bound glutamine residues and primary amines, including the .epsilon.-amino group of lysine residues in protein. Purified human erythrocyte TGase was found to have another activity, i.e., GTP hydrolysis. Treatment of the enzyme with iodoacetamide, a cysteine-directed reagent, caused a 94% loss of TGase activity within 8 min, but no significant loss of GTPase activity. Cys-277, a known residue which is selectively modified by iodoacetamide, was replaced with Ser by site-directed mutagenesis (C277S mutant) to assess the role of Cys-277 in the TGase/GTPase activities. Wild-type cDNA, coding for human endothelial cell TGase, and its C277S-mutated cDNA were cloned into a plasmid vector that contained a promotor from phage T7, and then expressed in Escherichia coli. The wild-type recombinant enzyme was indistinguishable from human erythrocyte TGase in mobility on a SDS-PAGE, immunoreactivity, and catalytic activities for TGase and GTPase. However, the recombinant enzyme was not blocked at the N-terminal alanine residue, as is the case in the naturally occurring erythrocyte enzyme. The C277S mutant enzyme showed no TGase activity, but had Km and kcat values for GTPase activity that were comparable to those of wild-type recombinant and natural erythrocyte enzymes. These results demonstrated that Cys-277 is essential for TGase activity, but not for GTPase activity, and that N-terminal blocking of tissue-type TGase is not crit. for either TGase or GTPase activities.

L3 ANSWER 51 OF 57 CAPLUS COPYRIGHT 2004 ACS on STN

1993:644285 Document No. 119:244285 Keratinocyte transglutaminase membrane anchorage: Analysis of site-directed mutants. Phillips, Marjorie A.; Qin, Qin; Mehrpouyan, Majid; Rice, Robert H. (Dep. Environ. Toxicol., Univ. California, Davis, CA, 95616-8588, USA). Biochemistry, 32(41), 11057-63 (English) 1993. CODEN: BICHAW. ISSN: 0006-2960.

AB Keratinocyte transglutaminase is anchored on the cytosolic side of the plasma membrane by fatty acid (palmitic acid) thioesterification near the amino terminus, a process which is seen to occur within 30 min of synthesis. The importance of a cluster of five cysteines (residues 47, 48, 50, 51, and 53) where acylation was presumed to occur is now demonstrated by site-directed mutagenesis. Transglutaminase mutants in which the cluster is deleted or the cysteines are all converted to alanine or serine are cytosolic. Partial replacement of the cluster, leaving two contiguous cysteines, is sufficient to confer membrane anchorage, while a single cysteine is only partially effective. As demonstrated with a sol. transglutaminase mutant, membrane anchorage confers susceptibility of the amino-terminal region to phorbol ester-stimulated phosphorylation. Attachment of 105 residues from the transglutaminase amino terminus to involucrin, a highly sol. protein, results in membrane anchorage of the hybrid protein. Attachment of the cysteine cluster alone does not result in membrane attachment of involucrin, but a 32-residue segment contg. this cluster is sufficient. Stable transfectants of human transglutaminase in mouse 3T3 cells are membrane-bound, indicating that fatty acid transacylation is not keratinocyte-specific.

L3 ANSWER 52 OF 57 CAPLUS COPYRIGHT 2004 ACS on STN

1993:407349 Document No. 119:7349 Use of transglutaminase in quality-improvement and processing of food proteins. Ikura, Koji; Sasaki, Ryuzo; Motoki, Masao (Fac. Agric., Kyoto Univ., Kyoto, 606, Japan). Comments on Agricultural and Food Chemistry, 2(6), 389-407 (English) 1992. CODEN: CACHEP. ISSN: 0892-2101.

AB A review with 36 refs. Transglutaminase catalyzes protein-modifying reactions such as amine-incorporation, crosslinking, and deamidation. The various attempts made to provide bases for a practical use of transglutaminase in quality-improvement and processing of food proteins are described. The mass prodn. of transglutaminase has become possible by establishing a system for prodn. of ***recombinant*** animal ***transglutaminase*** , and by screening a microorganism producing the enzyme. By use of the amine-incorporation catalyzed by transglutaminase,

desired amino acids were incorporated into food proteins to improve their nutritive and functional properties. A sol.-insol. interconvertible coenzyme was prepd. by incorporating the NAD amine deriv. into .alpha.s1-casein. Bovine milk caseins and soybean globulins were polymd. through the intermol. crosslinking catalyzed by the enzyme. The crosslinking between homogeneous and heterogeneous proteins often caused changes of their functional properties. Solns. of several food proteins at high concn. could be geled by transglutaminase. The gels were stable in denaturants and hot water. Emulsions prepd. with proteins and soybean oil were also converted into self-supporting gels by transglutaminase. Enzymic protein gelation was applied to the prepn. of a protein film.

L3 ANSWER 53 OF 57 CAPLUS COPYRIGHT 2004 ACS on STN
1993:402487 Document No. 119:2487 Cloning of human epidermal ***transglutaminase*** for ***recombinant*** manufacture of the enzyme. Yamanishi, Kyobumi; Fukushima, Shoji; Hirano, Jiro; Shii, Hiroshi (Adobansuto Sukin Risaachi Kenk, Japan). Jpn. Kokai Tokkyo Koho JP 05056785 A2 19930309 Heisei, 10 pp. (Japanese). CODEN: JKXXAF. APPLICATION: JP 1991-65034 19910328.

AB The cDNA for human epidermal transglutaminase (I) is cloned, sequenced, and expressed in Escherichia coli. From a cDNA library of human keratinocyte NHEK, the I cDNA was cloned with a DNA fragment (380 bp) amplified from the cDNA of human keratinocyte by polymerase chain reaction (PCR) using primers derived from a conserved region in the mammalian liver I, human coagulation factor XIIIa, and rabbit I. The I cDNA contg. one open reading frame was subcloned into pKK223-3 to construct pKHETG for expression of I cDNA in E. coli JM109. The I activity was obsd. in the occlusion body of recombinant E. coli JM109 cells.

L3 ANSWER 54 OF 57 CAPLUS COPYRIGHT 2004 ACS on STN
1992:443664 Document No. 117:43664 Polypeptides containing the fibrin-binding domain of fibronectin, their recombinant production, and their use in imaging and therapy. Vogel, Tikva; Levanon, Avigdor; Werber, Moshe; Guy, Rachel; Panet, Amos; Hartman, Jacob; Shaked, Hadassa (Bio-Technology General Corp., USA). PCT Int. Appl. WO 9117765 A1 19911128, 192 pp. DESIGNATED STATES: W: AU, BR, CA, FI, HU, JP, KR, NO, SU; RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LU, NL, SE. (English). CODEN: PIXXD2. APPLICATION: WO 1991-US3584 19910521. PRIORITY: US 1990-526397 19900521.

AB Polypeptides having amino acid sequences substantially present in the fibrin-binding domain (FBD) of human fibronectin are labeled with an imageable marker and used in imaging a thrombus or atherosclerotic plaque. Thrombolytic agents bound to the FBD polypeptides are also claimed. Wounds are treated with fusion products of the FBD polypeptide and a polypeptide comprising the cell-binding domain of human fibronectin. A human fibronectin cDNA library was prepd. and used in cloning and making various FBD polypeptides. The polypeptides were modified with DTPA and radiolabeled with 111In and shown to bind to preformed thrombi and thrombi in vivo. They gave a high thrombus:blood ratio of 80-200 after 24 h. The bacterial binding domain of fibronectin was shown to be sepd. from the FBD since a 31-kDa recombinant FBD polypeptide contg. the entire FBD (residues 1-262 of fibronectin) bound to Staphylococcus aureus, while 18.5 kDa and 12 kDa polypeptides contg. the 1st 154 and 109 amino acid residues of fibronectin, resp., did not. The 18.5 and 12 kDa polypeptides had a high covalent binding specificity for fibrin together with a narrower spectrum of activities and lower specificity for other ligands such as vascular components and bacteria than the 31 kDa protein which is advantageous for thrombus imaging.

L3 ANSWER 55 OF 57 CAPLUS COPYRIGHT 2004 ACS on STN
1990:438931 Document No. 113:38931 ***Recombinant*** manufacture of ***transglutaminase*** of Caviidae liver (MTGase) with Escherichia. Ikura, Koji; Sasaki, Ryuzo; Chiba, Hideo (Ajinomoto Co., Inc., Japan). Jpn. Kokai Tokkyo Koho JP 01300889 A2 19891205 Heisei, 8 pp. (Japanese). CODEN: JKXXAF. APPLICATION: JP 1988-132000 19880530.

AB A method for manufg. MTGase by cultivating recombinant E. coli is described. CDNA for MTGase was cloned from a guinea pig liver cDNA library and subsequently used to construct an expression plasmid pKTG1. The E. coli transformants were cultured and induced to produce MTGase detd. by Western blotting. Purifn. of the recombinant MTGase with monoclonal antibody to MTGase by affinity chromatog. was given. The

purified MTGase had a sp. activity of 1690 unit/mg .times. 104.

L3 ANSWER 56 OF 57 CAPLUS COPYRIGHT 2004 ACS on STN
1990:196870 Document No. 112:196870 Texturing of protein-rich foods with glutaminase-containing recombinant microorganisms. Costes, Claude; Frouin, Andre (Bongrain S. A., Fr.). PCT Int. Appl. WO 8907398 A1 19890824, 15 pp. DESIGNATED STATES: W: DK, JP, US. (French). CODEN: PIXXD2. APPLICATION: WO 1989-FR53 19890213. PRIORITY: FR 1988-1707 19880212.
AB Processed protein foods are textured using ***transglutaminases*** expressed by ***recombinant*** microorganisms to cross-link proteins and to incorporate otherwise rare amino acids (e.g. lysine, methionine). Beef 100 kg (10% lipid) ground by forcing through 3 mm holes was mixed with sugar 0.5 kg and 500 mL of a culture of recombinant Lactobacillus plantarum contg. the gene for guinea pig liver transglutaminase (109 cells/mL). Aliquots 100 g of this mixt. were conditioned for 10 h at 20.degree. and 15 h at 15.degree.. After refrigeration the meat had a texture comparable to steak.

L3 ANSWER 57 OF 57 CAPLUS COPYRIGHT 2004 ACS on STN
1990:135204 Document No. 112:135204 Expression of guinea pig liver transglutaminase cDNA in Escherichia coli. Amino-terminal N.alpha.-acetyl group is not essential for catalytic function of transglutaminase. Ikura, Koji; Tsuchiya, Yoichi; Sasaki, Ryuzo; Chiba, Hideo (Fac. Agric., Kyoto Univ., Kyoto, 606, Japan). European Journal of Biochemistry, 187(3), 705-11 (English) 1990. CODEN: EJBCAI. ISSN: 0014-2956.
AB As a first step of studies to elucidate the structure/function relationship of transglutaminase (EC 2.3.2.13), an expression plasmid, pKTG1, contg. a cDNA of guinea-pig liver transglutaminase between the NcoI and PstI sites of an expression vector, pKK233-2, was constructed, and the liver transglutaminase was produced as an unfused protein in E. coli. The purified recombinant enzyme was indistinguishable from natural liver transglutaminase in some structural properties such as mol. mass, amino acid compn., and amino- and carboxyl-terminal sequences. However, the .alpha.-amino group of the amino-terminal alanine residue of the ***recombinant*** ***transglutaminase*** was not acetylated as was that of the natural enzyme. Comparison of the recombinant enzyme with the natural one did not indicate significant differences in specific activity and apparent Km values for substrates in the histamine incorporation into acetyl .alpha.s1-casein. The sensitivity to activation by Ca2+ and the rate of catalyzed protein crosslinking were also similar between ***recombinant*** and natural ***transglutaminases*** . These results indicated that the N.alpha.-acetyl group in natural liver transglutaminase does not have a particular role in the catalytic function of this enzyme.

FILE 'CAPLUS' ENTERED AT 09:29:53 ON 05 JAN 2004

=> S TRANSGLUTAMINASE;S UREA;S GUANIDINE
          3958 TRANSGLUTAMINASE
           377 TRANSGLUTAMINASES
L1   4000 TRANSGLUTAMINASE
                (TRANSGLUTAMINASE OR TRANSGLUTAMINASES)

        191727 UREA
          8858 UREAS
L2   194427 UREA
                (UREA OR UREAS)

         30123 GUANIDINE
          2771 GUANIDINES
L3   30958 GUANIDINE
                (GUANIDINE OR GUANIDINES)

=> S L1 AND L2;S L1 AND L3
L4   58 L1 AND L2

L5   10 L1 AND L3

=> S L4,L5
L6   63 (L4 OR L5)

=>

=> D 1-63 CBIB ABS

L6   ANSWER 1 OF 63 CAPLUS COPYRIGHT 2004 ACS on STN
2003:932873 Experimental research of recombinant human growth hormone on obstructive jaundice. Wang, Tong; Jiang, Xiufeng; Wang, Chen; Kou, Zhimin (The Second Affiliated Hospital, Lanzhou Medical College, Lanzhou, 730030, Peop. Rep. China). Zhongguo Puwai Jichu Yu Linchuang Zazhi, 9(6), 408-411 (Chinese) 2002. CODEN: ZJLZFX. ISSN: 1007-9424. Publisher: Zhongguo Puwai Jichu Yu Linchuang Zazhi Bianji Weiyuanhui.
AB   New Zealand white rabbits were randomly divided into following groups: obstructive jaundice internal drainage plus recombinant human growth hormone (rhGH) group, obstructive jaundice internal drainage plus normal saline (NS) group, obstructive jaundice external drainage plus NS group, and obstructive jaundice external drainage plus rhGH group. After the establishment of obstructive jaundice model, rhGH of 0.2 IU/kg was s.c. injected in therapy groups twice a day. Meanwhile isovolume NS was used on the control groups. A full set of endotoxin (ET), tumor necrosis factor (TNF), sol. interleukin-2 receptor (sIL-2R) and nutritional status were estd. before the model establishment, 14 days after the model established, 14 days after internal and external drainage as well. Four days after internal and external drainage, body wt. of therapy groups was increased compared with control groups (P<0.05). Seven days and ten days after obstructive jaundice, blood sugar of therapy groups rised compared with control groups (P<0.05). The levels of albuminate (ALB), siderophilin (TFN) and prealbumin (PA) of therapy groups were all obsd. an increase 14 days after obstructive jaundice, and 14 days after internal and external drainage (P<0.01). Blood total cholesterol (CHO), low d. lipoprotein (LDL) and total bile acid (TBA) of therapy groups after 14 days of obstructive jaundice were increased apparently (P<0.05). Blood glutamic-oxaloacetic transaminase (AST), ***transglutaminase*** , total bilirubin, blood ***urea*** nitrogen (BUN), creatinine (Cr) and uric acid (UA) of therapy group after 14 obstructive jaundice days were also increased (P<0.05). Ca2+ of therapy groups 14 days after obstructive jaundice, 14 days after internal and external drainage rised as compared with control groups (P<0.05). However, K+, Na+ of therapy groups 14 days after external drainage decreased (P<0.05). An increasing tendency of sIL-2R was obsd. in control groups 14 days after obstructive jaundice (P<0.05) and ET, .alpha.-TNF, sIL-2R of control groups was decreased 14 days after internal and external drainage (P<0.01). After rhGH is used in obstructive jaundice and internal and external drainage, an improvement of

nutritional status and immunol. function can be obsd.

L6 ANSWER 2 OF 63 CAPLUS COPYRIGHT 2004 ACS on STN
2003:895063 Suwari gel properties as affected by ***transglutaminase*** activator and inhibitors. Benjakul, Soottawat; Visessanguan, Wonnop; Pecharat, Suttirak (Faculty of Agro-Industry, Department of Food Technology, Prince of Songkla University, Hat Yai, Songkhla, 90112, Thailand). Food Chemistry, 85(1), 91-99 (English) 2004. CODEN: FOCHDJ. ISSN: 0308-8146. Publisher: Elsevier Science.
AB Effect of ***transglutaminase*** activator and inhibitors on textural properties and crosslinking of myofibrillar proteins in suwari gel of surimi from four fish species, including bigeye snapper, threadfin bream, barracuda and bigeye croaker was investigated. Breaking force and deformation of suwari increased as calcium chloride added increased ($P<0.05$); however, a slight decrease was obsd. with an excessive amt. of addded calcium chloride. With addn. of calcium chloride, the concomitant decrease in soly. of suwari gel, in a mixt. of sodium dodecyl sulfate, ***urea*** and .beta.-mercaptoethanol, suggested increased non-disulfide covalent bond formation. Conversely, the addn. of ***transglutaminase*** inhibitors, including N-methylmaleimide (NEM), ammonium chloride and EDTA, resulted in a marked decrease in breaking force and deformation, esp. with increasing concn. The decrease in gel-forming ability was assocd. with the decrease in non-disulfide covalent crosslinking, as indicate by an increase in soly. and more myosin heavy chain (MHC) retained. The results indicate that endogenous ***transglutaminase*** played an essential role in setting, at high temp. (40 .degree.C), of surimi from tropical fish.

L6 ANSWER 3 OF 63 CAPLUS COPYRIGHT 2004 ACS on STN
2003:723059 Document No. 139:274144 Characterization of Periphilin, a Widespread, Highly Insoluble Nuclear Protein and Potential Constituent of the Keratinocyte Cornified Envelope. Kazerounian, Shideh; Aho, Sirpa (Department of Dermatology and Cutaneous Biology, Thomas Jefferson University, Philadelphia, PA, 19107, USA). Journal of Biological Chemistry, 278(38), 36707-36717 (English) 2003. CODEN: JBCHA3. ISSN: 0021-9258. Publisher: American Society for Biochemistry and Molecular Biology.
AB While keratinocytes go through the terminal differentiation and move toward the outer layers of epidermis, multiple proteins become sequentially incorporated into the cornified cell envelope. We have identified through yeast two-hybrid screening a novel protein, periphilin, interacting with periplakin, which is known as a precursor of the cornified cell envelope. Periphilin gene at chromosome 12q12 gives rise to multiple alternatively spliced transcripts. A monoclonal antibody detected the keratinocyte-specific periphilin isoform in undifferentiated keratinocytes in speckle-type nuclear granules and at the nuclear membrane, but in differentiated keratinocytes periphilin localized to the cell periphery and at cell-cell junctions, colocalizing there with periplakin. From cultured keratinocytes, periphilin was solubilized only after ***urea*** extn., indicating the highly insol. character of this protein. The nuclear localization, mediated through the N-terminal sequences of periphilin protein, is a prerequisite for the formation of insol. complexes. Although the globular N terminus of periphilin was necessary for the interaction with the periplakin tail, the keratinocyte-specific C terminus was responsible for the homodimerization. The C-terminal helical domain, composed of multiple heptad repeats, serves as a substrate for crosslinking by ***transglutaminases*** but also was specifically cleaved by caspase-5 in vitro. In conclusion, the localization pattern and insoly. of periphilin indicate that this novel protein is potentially involved in epithelial differentiation and contributes to epidermal integrity and barrier formation.

L6 ANSWER 4 OF 63 CAPLUS COPYRIGHT 2004 ACS on STN
2003:719270 Document No. 139:244701 Cross-linked antigen vaccines for treating infections, cancers, autoimmune diseases and Alzheimer's diseases. Chou, Szu-yi (USA). PCT Int. Appl. WO 2003074004 A2 20030912, 130 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG,

UZ, VC, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2003-US6661 20030303. PRIORITY: US 2002-PV361166 20020301; US 2002-PV363445 20020308; US 2002-231114 20020828; US 2002-231470 20020828; US 2002-231063 20020828; US 2002-231213 20020828; US 2002-231298 20020828.

AB Embodiments of the invention generally provide methods and compns. for producing polyvalent antigens, disease-specific antigens, ***transglutaminase*** -reactive compds., and recombinant ***transglutaminases*** . A method of producing a cross-linked compd. by a biol. agent is also provided. The invention provides a method for producing a cross-linked antigen and a method of using cross-linked products as antigens to immunize animals and induce strong immune responses. In addn., a method of producing an antigen specific for Alzheimer's disease is provided. Further, a method of producing a polyvalent antigen for two or more diseases is provided. Thus, compns. of antigens are prepd. and provided to immunize animals and induce strong immune responses. The invention provides purified recombinant ***transglutaminases*** reactive to a broad range of compds. and exhibit broad substrate activity. The recombinant ***transglutaminases*** are recombinant or chimeric ***transglutaminases*** of Streptomyces mobaraensis or Streptomyces cinnamoneus. Addnl., the invention provides a method of attaching one or more amino acid residues to a compd. to be reactive with ***transglutaminase*** , even for ***transglutaminase*** non-reactive compds.

L6 ANSWER 5 OF 63 CAPLUS COPYRIGHT 2004 ACS on STN

2003:434749 Document No. 139:18299 Generation of protein libraries using engineered suppressor tRNAs charged with desired amino acids in a coupled in vitro transcription/translation. Danielsen, Steffen (Novozymes A/S, Den.). PCT Int. Appl. WO 2003046195 A1 20030605, 42 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2002-DK805 20021202. PRIORITY: DK 2001-1778 20011130.

AB A method and kit for generating libraries of protein variants in vitro from a parent polynucleotide encoding a protein of interest, by introducing one or more stop codons in the parent polynucleotide, and performing sep. in vitro transcription and translation reactions with suppressor tRNA charged with at least two different amino acids, and a suitable cell-free in vitro expression system, are disclosed. The present invention offers the ability to introduce all 20 amino acids into any predefined position of any given polypeptide after only one site-directed mutagenesis step, wherein a stop codon is introduced into the encoding polynucleotide. An in vitro transcription/translation system using suppressor tRNA charged with different amino acids will enable subsequent prodn. of all the amino acid variants of the polypeptide simultaneously in parallel reactions. The gene encoding the haloperoxidase from C. verruculosa (rCvP) was cloned into a relevant expression vector (e.g. pRSET, InVitrogen). This placed the gene after the T7 promoter sequence followed by a Shine-Dalgarno ribosome binding site. A nonsense tag stop codon (amber codon) is inserted into the plasmid at position P173 of the CDS.

L6 ANSWER 6 OF 63 CAPLUS COPYRIGHT 2004 ACS on STN

2003:62252 Document No. 139:21173 Susceptibility of an industrial .alpha.-lactalbumin concentrate to cross-linking by microbial ***transglutaminase*** . Sharma, Ranjan; Zakora, Mila; Qvist, Karsten B. (Department of Dairy and Food Science, The Royal Veterinary and Agricultural University, Rolighedsvej 30, Frederiksberg C, DK-1958, Den.). International Dairy Journal, 12(12), 1005-1012 (English) 2002. CODEN: IDAJE6. ISSN: 0958-6946. Publisher: Elsevier Science B.V.

AB The susceptibility of an industrial .alpha.-lactalbumin conc. to crosslinking with a microbial ***transglutaminase*** from Streptoverticillium mobaraense was investigated. At a protein concn. of

0.5% w v-1, the max. crosslinking was obsd. at 50.degree., pH 5 and at 5 h of incubation time. Results from SDS-PAGE showed that most of the monomeric form of .alpha.-lactalbumin was converted to polymers too large to move into the gel matrix. Addn. of EDTA or SDS prior to the incubation of protein-enzyme mixt., further enhanced the ***transglutaminase*** reaction with the industrial .alpha.-lactalbumin. Results from reverse phase chromatog. indicated that crosslinking caused a broadening of the .alpha.-lactalbumin peak with little change in the av. hydrophobicity of the protein. In contrast to the reported results on pure .alpha.-lactalbumin, the industrial .alpha.-lactalbumin conc. showed considerable crosslinking with ***transglutaminase*** even without the redn. of the disulfide bonds. This difference was attributed to the partially unfolded secondary structures in the industrial .alpha.-lactalbumin conc.

L6 ANSWER 7 OF 63 CAPLUS COPYRIGHT 2004 ACS on STN
2003:48851 Document No. 139:68032 Effect of cross-linking with ***transglutaminase*** on the heat stability and some functional characteristics of sodium caseinate. Flanagan, J.; Gunning, Y.; FitzGerald, R. J. (Department of Life Sciences, University of Limerick, Limerick, Ire.). Food Research International, 36(3), 267-274 (English) 2003. CODEN: FORIEU. ISSN: 0963-9969. Publisher: Elsevier Science Ltd..
AB The effect of heating (140.degree., 0-60 min between pH 6.0 and 7.0) on the turbidity, pH 4.6 sol. amino group content and ***urea*** PAGE profiles of sodium caseinate (NaCN) and ***transglutaminase*** (TGase)-treated NaCN was detd. PH-dependent heat-induced changes in the turbidity and ***urea*** PAGE profiles of NaCN were initially attributed to casein aggregation followed by subsequent degrdn. on extended heating. Cross-linked NaCN samples (incubated with TGase at 20.degree., [E:S] of 1:50 and 1:20 for 185 min) were generally less turbid and had lower pH 4.6 sol. amino group content on heating than unmodified NaCN. The nitrogen soly. of cross-linked NaCN was improved at pH 2.0, 3.0 and 5.0. Some improvements in emulsifying activity index and stability of cross-linked NaCN were obsd. at pH 5.0 and 10.0. The improved heat stability and nitrogen soly. obsd. after TGase crosslinking may help extend the range of applications for NaCN.

L6 ANSWER 8 OF 63 CAPLUS COPYRIGHT 2004 ACS on STN
2003:48850 Document No. 139:68031 ***Transglutaminase*** -mediated setting in bigeye snapper Surimi. Benjakul, Soottawat; Visessanguan, Wonnop (Faculty of Agro-Industry, Department of Food Technology, Prince of Songkla University, Hat Yai, Songkhla, 90112, Thailand). Food Research International, 36(3), 253-266 (English) 2003. CODEN: FORIEU. ISSN: 0963-9969. Publisher: Elsevier Science Ltd..
AB Effect of setting induced by endogenous ***transglutaminase*** (TGase) in two species of bigeye snapper, Priacanthus tayenus and Priacanthus macracanthus, on gel properties and protein crosslinking was investigated. Setting at either 25 or 40 .degree.C, prior to heating at 90 .degree.C resulted in the increase in both breaking force and deformation of surimi from both species, particularly when setting time increased ($P < 0.05$). A decrease in soly. of surimi gels in a mixt. of sodium dodecyl-sulfate, ***urea*** and .beta.-mercaptoethanol suggested increased formation of non-disulfide covalent bonding which coincided with increased gel strength and the decrease in myosin heavy chain (MHC) polypeptide. The optimum conditions for setting of surimi sol was found to be 40 .degree.C for 2 h for P. tayenus and 25 .degree.C for 3 h for P. macracanthus. Assayed by monodancylcadaverine (MDC)-incorporation method, TGase from P. tayenus and P. macracanthus exhibited an optimum temp. at 40 and 25 .degree.C, resp. In addn., the breaking force and deformation of surimi from both species increased markedly with the addn. of calcium chloride, while they decreased considerably in the presence of EDTA, N-methylmaleimide and ammonium chloride. The results confirmed that endogenous ***transglutaminase*** played an important role in gel enhancement of surimi from both species of bigeye snapper.

L6 ANSWER 9 OF 63 CAPLUS COPYRIGHT 2004 ACS on STN
2003:5588 Document No. 138:200956 Highly Multilayered Urease Decomposes Highly Concentrated ***Urea*** . Kobayashi, Satoshi; Yonezu, Shinji; Kawakita, Hidetaka; Saito, Kyoichi; Sugita, Kazuyuki; Tamada, Masao; Sugo, Takanobu; Lee, William (Department of Materials Technology Faculty of Engineering, Chiba University, Chiba, 263-8522, Japan). Biotechnology

Progress, 19(2), 396-399 (English) 2003. CODEN: BIPRET. ISSN: 8756-7938. Publisher: American Chemical Society.

AB Urease was immobilized at a d. of 1.2 g of urease per g of a matrix via ion-exchange binding of urease to an anion-exchange polymer chain grafted onto a pore surface of a porous hollow-fiber membrane and subsequent crosslinking of urease with ***transglutaminase*** . ***Urea*** was hydrolyzed during the permeation of a ***urea*** soln., the concn. of which ranged from 2 to 8 M, through the pores of the resultant membrane with a thickness of approx. 1 mm. Quant. hydrolysis of 4 M ***urea*** was achieved at a permeation rate lower than 1 mL/h, i.e., a residence time longer than 5.1 min, at ambient temp. This performance is ascribed to convective transport of ***urea*** through the pores rimmed by the urease-immobilized polymer chains at a high d. Urease was denatured in the presence of ***urea*** at concns. higher than 6 M while hydrolyzing ***urea*** .

L6 ANSWER 10 OF 63 CAPLUS COPYRIGHT 2004 ACS on STN

2002:810334 Document No. 138:68890 In vitro refolding process of ***urea*** -denatured microbial ***transglutaminase*** without pro-peptide sequence. Yokoyama, Kei-ichi; Kunio, Ono; Ohtsuka, Tomoko; Nakamura, Nami; Seguro, Katsuya; Ejima, Daisuke (Central Research Laboratories, Ajinomoto Co. Inc., Kawasaki, Kanagawa, 210-8681, Japan). Protein Expression and Purification, 26(2), 329-335 (English) 2002. CODEN: PEXPEJ. ISSN: 1046-5928. Publisher: Elsevier Science.

AB Efficient refolding process of denatured mature microbial ***transglutaminase*** (MTG) without pro-peptide sequence was studied in the model system using ***urea*** -denatured pure MTG. Recombinant MTG, produced and purified to homogeneity according to the protocol previously reported, was denatured with 8M ***urea*** at neutral pH and rapidly dild. using various buffers. Rapid diln. with neutral pH buffers yielded low protein recovery. Redn. of protein concn. in the refolding soln. did not improve protein recovery. Rapid diln. with alk. buffers also yielded low protein recovery. However, diln. with mildly acidic buffers showed quant. protein recovery with partial enzymic activity, indicating that recovered protein was still arrested in the partially refolded state. Therefore, the authors further investigated the efficient refolding procedures of partially refolded MTG formed in the acidic buffers at low temp. (5.degree.). Although enzymic activity remained const. at pH 4, its hydrodynamic properties changed drastically during the 2 h after the diln. Titrn. of partially refolded MTG to pH 6 after 2 h of incubation at pH 4.0 improved the enzymic activity to a level comparable with that of the native enzyme. The same pH titrn. with incubations shorter than 2 h yielded less enzymic activity. Refolding trials performed at room temp. led to aggregation, with almost half of the activity yield obtained at 5.degree.. It was concluded that rapid diln. of ***urea*** -denatured MTG under acidic pH at low temp. results in specific conformations that can then be converted to the native state by titrn. to physiol. pH.

L6 ANSWER 11 OF 63 CAPLUS COPYRIGHT 2004 ACS on STN

2002:770357 Document No. 137:351838 Influence of ***transglutaminase*** treatment on some physico-chemical properties of milk. O'Sullivan, M. M.; Kelly, A. L.; Fox, P. F. (Department of Food Science, Food Technology and Nutrition, University College, Cork, Ire.). Journal of Dairy Research, 69(3), 433-442 (English) 2002. CODEN: JDRSAN. ISSN: 0022-0299. Publisher: Cambridge University Press.

AB ***Transglutaminase*** (TGase) is an enzyme that cross-links many proteins, including milk proteins. In this study, the effects of TGase on some physico-chem. properties of milk were studied. TGase-treated milk was not coagulable by rennet, which was due to failure of the primary (enzymic) stage of rennet action rather than the non-enzymic secondary phase. Dissocn. of TGase-treated casein micelles by ***urea*** or sodium citrate or removal of colloidal calcium phosphate by acidification and dialysis was reduced, presumably due to the formation of cross-links between the caseins. Casein micelles in TGase-treated milks were also resistant to high pressure treatment and to hydrolysis by plasmin. Results of the present study show that milk proteins are fundamentally modified by the action of TGase, which may have applications in the manuf. of functional proteins for use as novel food ingredients.

L6 ANSWER 12 OF 63 CAPLUS COPYRIGHT 2004 ACS on STN

2002:620717 Document No. 137:309803 Physicochemical and Nitrogen Solubility Properties of Bacillus Proteinase Hydrolysates of Sodium Caseinate Incubated with ***Transglutaminase*** Pre- and Post-hydrolysis. Flanagan, John; FitzGerald, Richard J. (Department of Life Sciences, University of Limerick, Limerick, Ire.). Journal of Agricultural and Food Chemistry, 50(19), 5429-5436 (English) 2002. CODEN: JAFCAU. ISSN: 0021-8561. Publisher: American Chemical Society.

AB Sodium caseinate (NaCN), hydrolyzed with Protamex, a Bacillus proteinase prepn., to 0.5, 1.3, and 17.5% degrees of hydrolysis, was incubated with ***transglutaminase*** (TGase) for 3, 42, and 290 min at enzyme/substrate ratios of 1, 1, and 10% (wt./wt.), resp., pre- and post-hydrolysis. The electrophoretic, reversed-phase high-performance liq. chromatog. (RP-HPLC) and nitrogen soly. profiles of the modified products were investigated. Combinations of hydrolysis and incubation with TGase generated products displaying novel physicochem. and nitrogen soly. properties. Significant changes in sodium dodecyl sulfate (SDS) and ***urea*** polyacrylamide gel electrophoresis profiles were apparent in the modified caseinate samples. Extensive TGase crosslinking resulted in polymers that were unable to enter the resolving gel during SDS polyacrylamide gradient gel electrophoresis. Extensive combined enzymic modification resulted in peptides eluting earlier on RP-HPLC than limited combined enzymic modification or limited hydrolysis. Combination of enzymic treatments resulted in significantly ($P < 0.005$) improved soly. around pH 4.6, compared to incubation with TGase or hydrolysis of NaCN alone.

L6 ANSWER 13 OF 63 CAPLUS COPYRIGHT 2004 ACS on STN

2002:348246 Document No. 137:78130 Physicochemical Study of .kappa.- and .beta.-Casein Dispersions and the Effect of Cross-Linking by ***Transglutaminase*** . de Kruif, C. G.; Tuinier, R.; Holt, C.; Timmins, P. A.; Rollema, H. S. (NIZO Food Research, Ede, 6710 BA, Neth.). Langmuir, 18(12), 4885-4891 (English) 2002. CODEN: LANGD5. ISSN: 0743-7463. Publisher: American Chemical Society.

AB The milk proteins .beta.- and .kappa.-casein are in a monomeric state below their crit. micelle concn. (cmc) and spontaneously self-assoc. above the cmc, at or above room temp. Small-angle neutron scattering (SANS) measurements at 5 g L-1 confirmed the high sp. vol. of these micelles, and 1H-NMR and calorimetric measurements showed that the polypeptide chains remain open and their side chains largely flexible in the assocd. state. The exptl. values of parameters used to describe both types of micelle were radius of gyration of 8 nm, scattering radius (in 2H2O) of 11 nm, and interaction radius close to 15 nm. The scattering radius is obtained by assuming a homogeneous sphere, and the interaction radius by assuming hard-sphere-like behavior. The micelle size, structure, and interaction radius were independent of concn. The interparticle structure factor of the micelles was detd. using a polydisperse hard-sphere model, which showed that the wave vector position of the peak of the interparticle structure factor was independent of concn. over the range 5-20 g L-1, consistent with effective hard-sphere behavior. The SANS and 1H-NMR expts. indicated that the .beta.-casein micelles were less compact and more dynamic than the .kappa.-casein micelles. The micelle dispersions were treated with the crosslinking enzyme ***transglutaminase*** . Scattering results showed that the crosslinking hardly influenced the structure or interaction of the micelles, indicating that there was little or no interparticle crosslinking. Addn. of 6 M ***urea*** did not change the structure of the cross-linked micelles, whereas addn. of the ***urea*** to the non-cross-linked micelles caused extensive dissocn.

L6 ANSWER 14 OF 63 CAPLUS COPYRIGHT 2004 ACS on STN

2002:54333 Document No. 137:5330 Gel properties of bigeye snapper (Priacanthus tayenus) surimi as affected by setting and porcine plasma proteins. Benjakul, Soottwat; Visessanguan, Wonnop; Srivilai, Chantira (Department of Food Technology, Prince of Songkla University, Songkhla, 90112, Thailand). Journal of Food Quality, 24(5), 453-471 (English) 2001. CODEN: JFQUD7. ISSN: 0146-9428. Publisher: Food & Nutrition Press, Inc..

AB Effects of setting temp., time, and addn. of porcine plasma protein (PPP) on gel properties of surimi from bigeye snapper (Priacanthus tayenus) were investigated. Breaking force and deformation of the surimi gels increased as the setting time and temp. increased. The gel preincubated at 35.degree.C for 90 min in the presence of 0.5% PPP, followed by cooking at 90.degree.C for 20 min showed the max. force and deformation. The

decrease in soly. of the resultant suwari and kamaboko gels in soln. contg. sodium dodecyl sulfate, ***urea*** and .beta.-mercaptoethanol suggested that gel enhancement was mainly mediated through the formation of non-disulfide covalent bonds catalyzed by both ***transglutaminase*** (TGase) in fish muscle and porcine plasma. Addn. of PPP slightly decreased the whiteness of the kamaboko gels.

L6 ANSWER 15 OF 63 CAPLUS COPYRIGHT 2004 ACS on STN

2002:31216 Document No. 136:90707 Skin conditioning compositions containing compounds for mimicking the effect of retinoic acid on skin. Granger, Stewart Paton; Scott, Ian Richard; Donovan, Robert Mark; Iobst-Teklits, Susanne; Licameli, Lisa (Unilever PLC, UK; Unilever NV; Hindustan Lever Limited). PCT Int. Appl. WO 2002002074 A2 20020110, 74 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2001-EP7234 20010625. PRIORITY: US 2000-PV215301 20000630.

AB A skin care product comprising about 0.001-10% of a retinoid, in combination with at least two retinoid boosters (0.0001-50%). Retinoid boosters are selected from fatty acid amides, carotenoids, flavonoids, non-cyclic fragrance compds., phospholipid analogs, ***ureas*** , phosphatidylcholines, phosphatidylethanolamines, sphingomyelins, fatty acids, linseed oil, elaidic acid, bifonazole, climbazole, clotrimazole, econazole, quercetin, coumarin, quinolines, isoquinolines, etc. A compn. according to the invention is intended primarily as a product for topical application to human skin, esp. as an agent for conditioning and smoothening the skin, and preventing or reducing the appearance of wrinkled or aged skin. In use, a small quantity of the compn. is applied to exposed areas of the skin, from a suitable container or applicator and, if necessary, it is then spread over and/or rubbed into the skin using the hand or fingers or a suitable device. For example, a synergistic inhibition of ***transglutaminase*** , as a marker of skin differentiation, was obsd. by retinol with various quaternary combinations of retinoid boosters, e.g., acetyl sphingosine, phosphatidylcholine, linoleic acid, and climbazole.

L6 ANSWER 16 OF 63 CAPLUS COPYRIGHT 2004 ACS on STN

2001:856991 Document No. 136:399219 ***Transglutaminase*** -dependent formation of protein aggregates as possible biochemical mechanism for polyglutamine diseases. Violante, V.; Luongo, A.; Pepe, I.; Annunziata, S.; Gentile, V. (Dipartimento di Biochimica e Biofisica, Seconda Universita' di Napoli (SUN), Naples, Italy). Brain Research Bulletin, 56(3/4), 169-172 (English) 2001. CODEN: BRBUDU. ISSN: 0361-9230. Publisher: Elsevier Science Inc..

AB A review. ***Transglutaminases*** (Enzyme Commission 2.3.2.13) are a large family of enzymes that show the common capacity to catalyze crosslinking of protein substrates. Some members of this family of enzymes are also capable of catalyzing other reactions important for the cell life. The distribution and the role of these enzymes have been widely studied in numerous cell types and tissues, but only recently their expression and functions started to be investigated in the central nervous system. One of the main biochem. properties of the ***transglutaminase*** enzymes is to form large protein aggregates that are insol. in all known protein detergents, such as ***urea*** , guanidinium, and SDS. Recently, the ***transglutaminase*** activity has been hypothesized to be involved in the pathogenetic mechanisms responsible for the formation of cellular inclusions present in Huntington disease and in all the other polyglutamine (polyQ) diseases hitherto identified, such as spinobulbar muscular atrophy or Kennedy disease, spinocerebellar ataxias (SCA-1, SCA-2, SCA-3 or Machado-Joseph disease, SCA-6 and SCA-7) and dentatorubropallidoluysian atrophy. In this review the authors describe the biochem. properties of the ***transglutaminase*** enzymes and some recent findings about the physiopathol. roles played by these enzymes in the central nervous system.

L6 ANSWER 17 OF 63 CAPLUS COPYRIGHT 2004 ACS on STN

2001:825648 Document No. 136:293770 Different arrangement of .epsilon.-(.gamma.-glutamyl)lysine cross-linking in Alaska pollock (Theragra chalcogramma) surimi proteins by Streptoverticillium and endogenous ***transglutaminases*** during suwari process. Sato, Kenji; Tanaka, Chie; Kotaru, Makoto; Yoshikawa, Hideki; Kawabata, Makoto; Ikeuchi, Tsuneo; Sato, Kenta; Nakamura, Yasushi; Ohtsuki, Kozo (Department of Food Sciences & Nutritional Health, Kyoto Prefectural University, Shimogamo Kyoto, 606-8522, Japan). Journal of Food Biochemistry, 25(5), 397-409 (English) 2001. CODEN: JFBIDW. ISSN: 0145-8884. Publisher: Food & Nutrition Press, Inc..

AB The objective of the present study is to compare the protein crosslinking reaction in Alaska pollock surimi that is catalyzed by a com. available microbial ***transglutaminase*** and by endogenous Alaska pollock ***transglutaminase*** . The endogenous ***transglutaminase*** was inhibited by EGTA and activated by CaCl2. The microbial ***transglutaminase*** was added to the salted surimi with and without EGTA and CaCl2. These surimi pastes were incubated at 25C up to 24 h followed by cooking at 90C. The resultant gels were fractionated into sol. and insol. (aggregate) fractions by SDS- ***urea*** extn. Compositional anal. revealed that the aggregate consisted predominantly of cross-linked myosin heavy chain. The distribution of .epsilon.-(.gamma.-glutamyl)lysine isopeptide in the sol. and aggregate fractions and peptide mapping analyses of the aggregate fraction demonstrate that the formation of isopeptide cross-links in Alaska pollock surimi proteins during suwari process differs when catalyzed by the microbial ***transglutaminase*** and endogenous ***transglutaminase*** .

L6 ANSWER 18 OF 63 CAPLUS COPYRIGHT 2004 ACS on STN

2001:499864 Document No. 135:97527 Methods of using primer molecules for enhancing the mechanical performance of tissue adhesives and sealants. Stedronsky, Erwin R. (Protein Polymer Technologies, Inc., USA). U.S. US 6258872 B1 20010710, 13 pp., Cont.-in-part of U.S. Ser. No. 879,564. (English). CODEN: USXXAM. APPLICATION: US 1998-102521 19980622. PRIORITY: US 1997-879564 19970620.

AB The present invention concerns novel methods for enhancing the mech. performance of tissue adhesives and sealants which comprises employing a primer mol. in assocn. with a tissue adhesive or sealant, wherein the primer mol. serves to enhance the strength of the interface between the tissue and the adhesive matrix. The primer mols. described herein function to interact with a protein present in the tissue, thereby altering its characteristics to make it more amenable to bonding with the adhesive matrix. Primer mols. may be applied to the tissue independently from the tissue adhesive or sealant or may be mixed with the tissue adhesive or sealant prior to application to the tissue. In an attempt to identify novel methods for enhancing the mech. performance of tissue adhesives by increasing the strength of the adhesive matrix/tissue-assocd. protein interface, lap shear tensile testing was performed with a variety of different combinations of adhesive matrix precursors, crosslinkers, primers, methods of primer prepn., methods of applying the primers, dopes, methods of applying the dopes and curing agents. The results from these expts. are presented wherein each of the above described variables are indicated by their codes.

L6 ANSWER 19 OF 63 CAPLUS COPYRIGHT 2004 ACS on STN

2001:342355 Document No. 135:91581 Improvement of shark type I collagen with microbial ***transglutaminase*** in ***urea*** . Nomura, Yoshihiro; Toki, Shinzi; Ishii, Yasuhiro; Shirai, Kunio (Applied Protein Chemistry, Faculty of Agriculture, Tokyo University of Agriculture and Technology, Tokyo, 183-8509, Japan). Bioscience, Biotechnology, and Biochemistry, 65(4), 982-985 (English) 2001. CODEN: BBBIEJ. ISSN: 0916-8451. Publisher: Japan Society for Bioscience, Biotechnology, and Agrochemistry.

AB In the presence of ***urea*** , type I collagen could form a gel with crosslinks with microbial ***transglutaminase*** (MTGase). Collagen self-assembly was accelerated with the addn. of MTGase. The proportion of reconstructed collagen fibrils was raised with the addn. of MTGase. MTGase-treated collagen gel remained gelled at high temps. at which collagen denatured. By treatment with MTGase, collagen could form the gel under impossible condition to collagen self-assembly, and that denaturation temp. was raised.

L6 ANSWER 20 OF 63 CAPLUS COPYRIGHT 2004 ACS on STN
2000:776898 Document No. 134:40326 Molecular chaperonic function of C-reactive protein induced by heating in HT-29 human colon carcinoma cells. Lee, Soo Young; Jung, Hyun-Jung; Kim, Hyun-Soo; Lee, Seung-Chul; Lee, Si-Back; Joe, Jae-Hoon; Kim, You-Mie (Department of Natural Sciences, Chemistry Section, College of Medicine, The Catholic University of Korea, Seoul, 137-701, S. Korea). Journal of Biochemistry and Molecular Biology, 33(5), 407-411 (English) 2000. CODEN: JBMBE5. ISSN: 1225-8687. Publisher: Springer-Verlag Singapore Pte. Ltd..

AB The effect of heat shock, or all-trans retinoic acid, on the expression of the C-reactive protein mRNA in the HT-29 human colon carcinoma cells, as well as the functional role of the C-reactive protein as a mol. chaperone, were studied. The expression level of the C-reactive protein mRNA in the HT-29 cells was increased time-dependently when exposed to heat-shock, and dose-dependently when treated with all-trans-Retinoic acid. The activities of ***transglutaminase*** C and K in the HT-29 cells were significantly increased when treated with all-trans retinoic acid. The C-reactive protein prevented thermal aggregation of the citrate synthase and stabilized the target enzyme, citrate synthase. The C-reactive protein promoted functional refolding of the ***urea*** -denatured citrate synthase up to 40-70%. These results suggest that the C-reactive protein, which is induced in human colon carcinoma cells, when heated or treated with all-trans-Retinoic acid has in a part functional activity of the mol. chaperone.

L6 ANSWER 21 OF 63 CAPLUS COPYRIGHT 2004 ACS on STN
2000:475778 Document No. 133:101387 Process for producing enzymatically active microbial ***transglutaminase*** via two step refolding. Yokoyama, Keiichi; Ono, Kunio; Ejima, Daisuke (Ajinomoto Co., Inc., Japan). PCT Int. Appl. WO 2000040706 A1 20000713, 74 pp. DESIGNATED STATES: W: AU, BR, CA, CN, JP, US; RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE. (Japanese). CODEN: PIXXD2. APPLICATION: WO 1999-JP7250 19991224. PRIORITY: JP 1998-373131 19981228.

AB A method of producing enzymically active microbial ***transglutaminase*** (TGase) by subjecting ***transglutaminase*** in a denatured state to a refolding process involving at least the following steps (a) and (b): (a) the step of forming an intermediate structure, showing the enzymic activity in an aq. medium under acidic conditions, and (b) the step of forming a higher-order structure, showing the enzyme activity in an aq. medium at a neutral pH value. Cysteine thiol is preferably in a free reduced state, and TGase in soln. during the step (a). The process may addnl. include the treatment with agent that facilitate the higher-order structure formation, denaturing agent, and sepn. of inactive enzyme as aggregate. The intermediate structure should have 30 ~ 70% mol. ellipticity in near UV CD spectra. These are accomplished by adjusting the pH of enzyme contg. mildly acidic soln. to the neutral range.

L6 ANSWER 22 OF 63 CAPLUS COPYRIGHT 2004 ACS on STN
2000:466956 Document No. 133:248722 Overproduction of microbial ***transglutaminase*** in Escherichia coli, in vitro refolding, and characterization of the refolded form. Yokoyama, Kei-Ichi; Nakamura, Nami; Seguro, Katsuya; Kubota, Kouji (Central Research Laboratories, Ajinomoto Co. Inc., Kanagawa, 210-0801, Japan). Bioscience, Biotechnology, and Biochemistry, 64(6), 1263-1270 (English) 2000. CODEN: BBBIEJ. ISSN: 0916-8451. Publisher: Japan Society for Bioscience, Biotechnology, and Agrochemistry.

AB The Streptoverticillium ***transglutaminase*** (MTG) gene, synthesized previously for yeast expression, was modified and resynthesized for overexpression in E. coli. A high-level expression plasmid, pUCTRPMTG-02(+), was constructed. Furthermore, to eliminate the N-terminal methionine, pUCTRPMTGD2 was constructed. Cultivation of E. coli transformed with pUCTRPMTG-02(+) or pUCTRPMTGD2 yielded a large amt. of MTG (200.apprx.300 mg/L) as insol. inclusion bodies. The N-terminal amino acid residue of the expressed protein was methionine or serine (the second amino acid residue of the mature MTG sequence), resp. Transformed E. coli cells were disrupted, and collected pellets of inclusion bodies were solubilized with 8 M ***urea*** . Rapid diln. treatment of solubilized MTG restored the enzymic activity. Refolded MTG, purified by ion-exchange chromatog., which had an N-terminal methionine or serine residue, showed activity equiv. to that of native MTG. These results

indicated that recombinant MTG could be produced efficiently in E. coli.

L6 ANSWER 23 OF 63 CAPLUS COPYRIGHT 2004 ACS on STN
1999:156403 Document No. 130:206690 Cathepsin L isolated from mammalian epidermis and its use in cosmetic and pharmaceutical compositions. Bernard, Dominique; Kermici, Michel; Bernard-Bourboulon, Marie-Alix (L'Oreal, Fr.). Eur. Pat. Appl. EP 899330 A1 19990303, 16 pp. DESIGNATED STATES: R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO. (French). CODEN: EPXXDW. APPLICATION: EP 1998-402037 19980811. PRIORITY: FR 1997-10818 19970829.
AB A cathepsin L isolated from mammalian skin, said protease modulating intercorneocyte assocn., is disclosed. The cathepsin L may be used in cosmetic compns. for desquamation or in pharmaceutical compns. for treatment of desquamation disorders, such as hyperkeratosis and psoriasis. Thus, a cathepsin L was isolated from human skin. It has a mol. wt. between 15-32 kilodaltons and a pI of 6-9. It degrades corneodesmosine.

L6 ANSWER 24 OF 63 CAPLUS COPYRIGHT 2004 ACS on STN
1998:289203 Document No. 129:27170 Properties of films produced by crosslinking whey proteins and 11S globulin using ***transglutaminase*** . Yildirim, M.; Hettiarachchy, N. S. (Dept. of Food Science, Univ. of Arkansas, Fayetteville, AR, 72704, USA). Journal of Food Science, 63(2), 248-252 (English) 1998. CODEN: JFDSAZ. ISSN: 0022-1147. Publisher: Institute of Food Technologists.
AB ***Transglutaminase*** (TG) was used to produce films from whey protein isolate, soybean 11S globulin and a mixt. of the two (1:1, wt/wt). Soly. of TG cross-linked films was lower than that of control films at pH 3, 4, 6 and 8. Soly. of control films in 6.6M ***urea*** and in 10% SDS was higher than that of TG cross-linked films. Hydrolysis of control and TG cross-linked films by trypsin and .alpha.-chymotrypsin was similar after 24h incubation. Mean thickness of films ranged from 69 to 77 .mu.m and there were no differences among thicknesses. Av. tensile strength values of TG cross-linked films were two times greater than those of the homologous controls.

L6 ANSWER 25 OF 63 CAPLUS COPYRIGHT 2004 ACS on STN
1997:812444 Document No. 128:60811 Non-thermally induced gel of proteins. Katsuta, Keiko (Sch. Hum. Life Environ., Nara Women's Univ., Nara, 630, Japan). Nippon Shokuhin Kagaku Kogaku Kaishi, 44(12), 917-924 (Japanese) 1997. CODEN: NSKKEF. ISSN: 1341-027X. Publisher: Nippon Shokuhin Kagaku Kogakkai.
AB A review with 70 refs., on studies on non-thermally induced food protein gels, discussing rennet-induced gels, gelation by ***transglutaminase*** , thiol-dependent gels, ***urea*** -induced gel, polylysine-induced gels, and thermally induced gels. The gelation mechanism of proteins is also discussed.

L6 ANSWER 26 OF 63 CAPLUS COPYRIGHT 2004 ACS on STN
1997:433942 Document No. 127:172921 Tridegin, a new peptidic inhibitor of factor XIIIa, from the blood-sucking leech Haementeria ghilianii. Finney, Sarah; Seale, Lisa; Sawyer, Roy T.; Wallis, Robert B. (Biopharm (U.K.) Ltd., Dyfed, SA4 1XB, UK). Biochemical Journal, 324(3), 797-805 (English) 1997. CODEN: BIJOAK. ISSN: 0264-6021. Publisher: Portland Press.
AB 1. Crude salivary gland ext. of the giant Amazon leech, Haementeria ghilianii, contains an inhibitor of plasma factor XIIIa. 2. The inhibitory agent was purified to homogeneity by anion-exchange, cation-exchange, gel-filtration and reverse-phase chromatog. to yield a single band on SDS-PAGE with an apparent mol. mass of 7.3 kDa. It has been named tridegin. 3. Micro-sequencing of proteolytic fragments showed tridegin to be a peptide of 66 amino acids. The sequence is unique with little similarity to other leech-derived proteins. 4. Inhibition of plasma factor XIIIa activity was confirmed by four independent methods: tridegin increased the soly. of fibrin clots in ***urea*** , inhibited ammonia produced from the incorporation of ethylamine into casein, inhibited the incorporation of 5'-(biotinamido)pentylamine into casein and prevented .gamma.-dimer formation in clotting fibrinogen. 5. The IC50 of tridegin (approx. 9.2 nM) is very close to the concn. of factor XIIIa used in the assay and in fact depends on its concn. This is the most potent inhibitor of factor XIIIa yet described. 6. Tridegin also inhibits platelet factor XIIIa (factor XIIIAa) with a similar potency to that of the plasma enzyme. 7. Tridegin also inhibits tissue

transglutaminase but with lower potency and independently of the enzyme concn. 8. Tridegin appears to be specific for ***transglutaminases*** , since it has no effect on the coagulation times of human plasma, on thrombin or factor Xa. Moreover it has no effect on other thiol-contg. enzymes and has no ability to digest fibrinogen or cleave the isopeptide substrate, L-.gamma.-glutamyl-4-nitroanilide.

L6 ANSWER 27 OF 63 CAPLUS COPYRIGHT 2004 ACS on STN
1997:16869 Document No. 126:102504 The association of tissue ***transglutaminase*** with human recombinant tau results in the formation of insoluble filamentous structures. Appelt, Denah M.; Balin, Brian J. (Department of Anatomy and Neurobiology, Allegheny University of the Health Sciences, Broad and Vine Streets, NCB RM 5602, MS #435, Philadelphia, PA, 19102-1192, USA). Brain Research, 745(1,2), 21-31 (English) 1997. CODEN: BRREAP. ISSN: 0006-8993. Publisher: Elsevier.
AB To det. possible mechanisms by which NFTs (neurofibrillary tangles) are formed in Alzheimer's disease (AD), the authors investigated the ability of tissue ***transglutaminase*** (TGase) to convert human recombinant tau proteins into insol. filamentous structures. TGase derived from guinea pig liver was activated by calcium to catalyze the in vitro crosslinking of the largest sol. recombinant tau isoform (htau40) into insol. complexes as detd. by electrophoresis following incubation in 4 M ***urea*** and SDS. The TGase-catalyzed formation of these insol. complexes occurred within 15 min to 24 h and the decreased migration of the insol. material correlated with increased calcium concns. ranging from 2 mM to 50 mM when analyzed electrophoretically. TGase-treated human recombinant tau formed filamentous structures in vitro that were immunoreactive with antibodies to tau and TGase. These structures retained the insol. characteristics typical of AD PHF (paired helical filaments)/NFTs. Immunolabeling with the TGase antibody revealed that TGase is assocd. with the filaments formed from human recombinant tau in vitro as well as with PHFs isolated from NFTs from AD brains. These novel findings support an in vitro model for investigating the biophys. changes that occur in converting sol. tau proteins into an insol. matrix consistent with the insol. PHFs/NFTs which may contribute to neuronal degeneration and cell death in the AD brain.

L6 ANSWER 28 OF 63 CAPLUS COPYRIGHT 2004 ACS on STN
1996:487599 Document No. 125:191133 Analysis of chorion hardening of eggs of rainbow trout, Oncorhynchus mykiss. Iuchi, Ichiro; Ha, Chang-Rak; Sugiyama, Hitoshi; Nomura, Kohji (Life Sci. Inst., Sophia Univ., Tokyo, 102, Japan). Development, Growth & Differentiation, 38(3), 299-306 (English) 1996. CODEN: DGDFA5. ISSN: 0012-1592. Publisher: Blackwell.
AB We estd. changes of chorion hardness of rainbow trout (O. mykiss) egg by the use of 3 parameters, namely increase of resistance of an egg to rupture by extraneously applied pressure, decrease of soly. of chorion proteins in 8M ***urea*** , and a change in the content of .gamma.-glutamyl-.epsilon.-lysine crosslink. Unfertilized egg chorions became hardened after egg activation,. During chorion hardening, 49-, 56-, and 65-kDa protein components of the chorion gradually disappeared, high-mol.-wt. intermediates (113, 160-170, and >250 kDa) were newly formed and, finally, all components became undetectable by SDS-PAGE. Chorion hardening was inhibited by the incorporation of monodansylcadaverine, a competitive inhibitor for ***transglutaminase*** (TGase), into the chorions. TGase activity was detected in unfertilized eggs and localized in the chorion fraction rather than in the ooplasmic fraction. The findings suggest that chorion hardening depends upon polymn. of the chorion components by TGase-dependent .gamma.-Glu-.epsilon.-Lys crosslink formation.

L6 ANSWER 29 OF 63 CAPLUS COPYRIGHT 2004 ACS on STN
1996:139498 Document No. 124:224547 The bacterial over-expression of C-terminal domain of human ***transglutaminase*** 2. Bae, Chang-Dae; Park, Won-Man; Choi, Eun-Young; Hong, Kyeong-Man; Jung, Sung-Won; Juhn, Steven K.; Lee, Tong-Ho; Park, Won-Jae; Kim, Soo-Youl (College Medicine, Hallym University, Kangwon, S. Korea). Korean Journal of Biochemistry, 27(4), 239-45 (English) 1995. CODEN: KJBID3. ISSN: 0378-8512. Publisher: Korean Biochemical Society.
AB The authors have designed the construction of a bacterial overexpression system with human ***transglutaminase*** (TGase) 2 C-terminal insert in a pET-17xb vector. After the correct colony was selected, the target

protein was induced by isopropyl-1-thio-.beta.-D-galactopyranoside. Almost 70% of the total expressed proteins were presented in inclusion body. After washing with high salt 8 times to get rid of sol. impurities, the insol. pellet was dissolved in 6 M ***urea*** soln. That soln. was chromatographed through a DEAE-cellulose column equilibrated with 6 M ***urea*** , pH 7.5, and the pooled sample contg. the expressed TGase 2 protein was dialyzed against acidic buffer (pH 5.0) or basic buffer (pH 9.0) contg. various concn. (10 .mu.M-10 mM) of dithiothreitol. By this procedure, the authors obtained more than 95% pure sol. form of expressed protein.

L6 ANSWER 30 OF 63 CAPLUS COPYRIGHT 2004 ACS on STN
1995:923119 Document No. 124:3744 Biochemical, structural, and ***transglutaminase*** substrate properties of human loricrin, the major epidermal cornified cell envelope protein. Candi, Eleonora; Melino, Gerry; Mei, Giampiero; Tarcsa, Edit; Chung, Soo-Il; Marekov, Lyuben N.; Steinert, Peter M. (Skin Biol. Branch, Natl. Inst. Health, Bethesda, MD, 20892-2775, USA). Journal of Biological Chemistry, 270(44), 26382-90 (English) 1995. CODEN: JBCHA3. ISSN: 0021-9258. Publisher: American Society for Biochemistry and Molecular Bio logy.
AB Loricrin is the major protein of the cornified cell envelope of terminally differentiated epidermal keratinocytes which functions as a phys. barrier. In order to understand its properties and role in cornified cell envelope, we have expressed human loricrin from a full-length cDNA clone in bacteria and purified it to homogeneity. We have also isolated loricrin from newborn mouse epidermis. By CD and fluorescence spectroscopy, the in vivo mouse and bacterially expressed human loricrins possess no .alpha. or .beta. structure but have some organized structure in soln. assocd. with their multiple tyrosines and can be reversibly denatured by either ***guanidine*** hydrochloride or temp. The ***transglutaminase*** (TGase) 1, 2, and 3 enzymes expressed during epidermal differentiation utilized loricrin in vitro as a complete substrate, but the types of crosslinking were different. The TGase 3 reaction favored certain lysines and glutamines by forming mostly intrachain cross-links, whereas TGase 1 formed mostly large oligomeric complexes by interchain cross-links involving different lysines and glutamines. Together, the glutamines and lysines used in vitro are almost identical to those seen in vivo. The data support a hypothesis for the essential and complementary roles of both TGase 1 and TGase 3 in crosslinking of loricrin in vivo. Failure to cross-link loricrin by TGase 1 may explain the phenotype of lamellar ichthyosis, a disease caused by mutations in the TGase 1 gene.

L6 ANSWER 31 OF 63 CAPLUS COPYRIGHT 2004 ACS on STN
1995:837915 Document No. 123:249324 Association of ***transglutaminase*** with the reconstituted keratin filaments isolated from rat vaginal epithelial cells. Vijayalakshmi, V.; Gupta, P. D. (Centre for Cellular and Molecular Biology, Hyderabad, 500 007, India). Epithelial Cell Biology, 3(4), 168-74 (English) 1994. CODEN: ECBIEP. ISSN: 0940-9912. Publisher: Springer.
AB The authors report a novel assocn. of the calcium dependent crosslinking enzyme, ***transglutaminase*** (TGase) with the ***urea*** sol. reconstituted keratin filaments (RKF) isolated from the rat vaginal epithelial cells (VEC). This was ascertained by measuring the activity using 14C-spermidine incorporation and also by an increase in keratin filament aggregation by the addn. of only TGase cofactor-calcium. These events were specifically inhibited by the treatment of calcium chelator, EDTA at a concn. >2 mM as well as by pretreating the RKF with histamine, a TGase substrate inhibitor. The assocn. was also exemplified by immunoblotting anal. where a specific and preferential polypeptide of mol. wt. 58 kDa cross-reacted with TGase antibody amongst the other keratins. This phenomenon was not seen in the keratins isolated from skin, a non-targeting tissue for estradiol action.

L6 ANSWER 32 OF 63 CAPLUS COPYRIGHT 2004 ACS on STN
1995:653859 Document No. 123:54466 Contribution of SS bonds to the elasticity of actomyosin gel in which coexisting ***transglutaminase*** was inactivated. Niwa, Eiji; Inuzuka, Kumiko; Nowsad, Alam Akm; Liu, Dajia; Kanoh, Satoshi (Faculty Bioresources, Mie University, Mie, 514, Japan). Fisheries Science, 61(3), 438-40 (English) 1995. CODEN: FSCIEH. ISSN: 0919-9268. Publisher: Japanese Society of Fisheries Science.
AB The contribution of SS bonds to the elasticity of suwari gel from various

actomyosins was investigated, in which a coexisting ***transglutaminase*** was inactivated. The actomyosins, with SH groups blocked (SH-blocked AM) or unblocked (SH-unblocked AM), were prepd., resp., by shaking the starting actomyosins with and without N-ethylmaleimide in 8 M ***urea*** . After removal of ***urea*** by dialysis, they were measured for ***transglutaminase*** activity, total SH content, and suwari gel forming ability. The ***transglutaminase*** activity of both the actomyosins became zero by the above treatment with ***urea*** . The suwari gel prepd. by setting SH-unblocked AM paste at 40.degree.C for 1 h was considerably higher in the breaking force than the gel from the paste of the SH-blocked one. By setting the AM paste, total SH content was somewhat decreased in the former, but scarcely changed in the latter.

L6 ANSWER 33 OF 63 CAPLUS COPYRIGHT 2004 ACS on STN
1994:698393 Document No. 121:298393 Protein alterations in age-related cataract associated with a persistent hyaloid vascular system in senescence-accelerated mouse (SAM). Ashida, Yasushi; Takeda, Toshio; Hosokawa, Masanori (Dep. of Senescence Biology, Kyoto Univ., Kyoto, 606, Japan). Experimental Eye Research, 59(4), 467-74 (English) 1994. CODEN: EXERA6. ISSN: 0014-4835. Publisher: Academic.
AB The occurrence of age-related cataract assocd. with a persistent hyaloid vascular system is the most prominent feature in SAMP9, an inbred strain of senescence-accelerated mouse. To examine the cataractogenesis, the authors analyzed protein changes in the process of cataract formation in the lens. The cataractous lenses showed a striking decrease in water-sol. protein content in contrast to increases in the amt. of water-insol. protein. SDS-PAGE and Western blots of water-sol. protein in the cataractous lenses showed addnl. high mol. wt. .beta.-crystallin proteins of about 43 kDa, concomitant with decreased amts. of 29-kDa and 31-kDa .beta.-crystallins and 21-kDa .gamma.-crystallin, as compared with findings in normal lenses. Although there was no apparent difference between the patterns of SDS-PAGE of ***urea*** -sol. and ***urea*** -insol. proteins isolated from cataractous and normal lenses, slightly increased reactivity of bands around 43 kDa against anti-.beta.-crystallin antibody was obsd. in cataractous lenses. The calcium content was elevated and activity of ***transglutaminase*** was increased in the cataractous lenses. While the mol. wt. of .beta.-crystallin polymers cross-linked in vitro by exogenous ***transglutaminase*** was not completely compatible with those of high mol. wt. .beta.-crystallins obsd. in the cataractous lenses, these findings do suggest the contribution of this enzyme to prodn. of high mol. wt. .beta.-crystallins and to insolubilization of these proteins in the cataractous lenses in SAMP9.

L6 ANSWER 34 OF 63 CAPLUS COPYRIGHT 2004 ACS on STN
1994:679286 Document No. 121:279286 Quality of kamaboko gel prepared from walleye pollack surimi with an additive containing ***transglutaminase*** . Abe, Yoichi (Abe Jyuro Co., Ltd., Hokkaido, 093, Japan). Nippon Suisan Gakkaishi, 60(3), 381-7 (Japanese) 1994. CODEN: NSUGAF. ISSN: 0021-5392.
AB . Kamaboko gels were prepd. by adding 0.3% food additive contg. ***transglutaminase*** in order to set, at a no. of different temps., frozen salt-ground meat of walleye pollack surimi. The quality of the kamaboko gels thus formed was compared to kamaboko gels formed without the additive. The breaking strength of the kamaboko gel formed with the additive was much higher, whereas the breaking strain remained at a fairly low level. However, gelling of salt-ground meat in the presence of the additive varied somewhat depending on the setting temp. In addn., soly. of the kamaboko gel formed with the same additive into SDS (sodium dodecylsulfate)- ***urea*** -mercaptoethanol mixt. was much lower as setting time progressed, while the kamaboko gel without the additive was easily sol. into the same medium. These results indicate that kamaboko gel prepd. in the presence of ***transglutaminase*** additive is different from that product without the additive.

L6 ANSWER 35 OF 63 CAPLUS COPYRIGHT 2004 ACS on STN
1994:570851 Document No. 121:170851 Estradiol-regulated transamidation of keratins by vaginal epithelial cell ***transglutaminase*** . Vijayalakshmi, V.; Gupta, P. D. (Centre Cellular & Mol. Biology, Hyderabad, 500 007, India). Experimental Cell Research, 214(1), 358-66 (English) 1994. CODEN: ECREAL. ISSN: 0014-4827.

AB The authors have obsd. a marked increase in the activity of ***transglutaminase*** (EC 2.3.2.13) in rat vaginal epithelial cells in a time-dependent manner during estradiol-induced terminal differentiation. The increased ***transglutaminase*** activity facilitates a post-translational modification, transamidation of keratins by the formation of an isopeptide .SIGMA.(.tau.-glutamyl)lysine. This isopeptide was recovered from the ***urea*** -sol. and -insol. fractions of keratins. The formation of .SIGMA.(.tau.-glutamyl)lysine was significantly reduced in rats primed with progesterone or tamoxifen-estradiol. These in vivo expts. were further confirmed by in vitro studies using the reconstituted keratin filaments, which demonstrated a remarkable acceleration in the formation of covalent cross-links mediated by vaginal epithelial cell ***transglutaminase*** obtained from rats primed with estradiol. By specifically modifying the lysine residues of the keratins with 2,4-pentanedione the aggregation of keratin filaments was inhibited. These findings reflect that the vaginal epithelial cell ***transglutaminase*** obtained from rats which is regulated by estradiol plays a key role in the process of terminal differentiation of rat vaginal epithelial cells.

L6 ANSWER 36 OF 63 CAPLUS COPYRIGHT 2004 ACS on STN
1994:238968 Document No. 120:238968 Identification of chlorophyll-a/b proteins as substrates of ***transglutaminase*** activity in isolated chloroplasts of Helianthus tuberosus L.. Del Duca, S.; Tidu, V.; Bassi, R.; Esposito, C.; Serafini-Fracassini, D. (Dip. Biol. Evol. Sper., Univ. Bologna, Bologna, I-40126, Italy). Planta, 193(2), 283-9 (English) 1994. CODEN: PLANAB. ISSN: 0032-0935.

AB Endogenous substrates of ***transglutaminase*** (TGase; EC 2.3.2.13) have been identified in chloroplasts of Helianthus tuberosus leaves. The activity of TGase is Ca2+- and light-stimulated and catalyzes the incorporation of polyamines into thylakoid and stromal proteins. These proteins were sepd. by two-dimensional gel electrophoresis (first dimension: Deriphat-PAGE; second dimension: SDS- ***urea*** -PAGE) and Western-blotted. The thylakoid proteins were recognized by polyclonal antibodies as apoproteins of the chlorophyll-a/b antenna complex (LHCII, CP24, CP26 and CP29); a stromal protein was recognized by antibodies as the large subunit of ribulose-1,5-bisphosphate carboxylase/oxygenase. A possible localization of the acyl donor site for CP26 is proposed. A comparative anal. of polyamine incorporation into trichloroacetic-acid-precipitable material indicated that spermidine was a preferential acyl-acceptor substrate with respect to putrescine. The nature of the substrates, together with the light stimulation, support the working hypothesis of a possible role of TGase in regulating the light-harvesting function.

L6 ANSWER 37 OF 63 CAPLUS COPYRIGHT 2004 ACS on STN
1993:667985 Document No. 119:267985 ***Transglutaminase*** catalyzes the formation of sodium dodecyl sulfate-insoluble, Alz-50-reactive polymers of .tau.. Dudek, Serena M.; Johnson, Gail V. W. (Dep. Psychiatry Behav. Neurobiol., Univ. Alabama, Birmingham, AL, 35294-0017, USA). Journal of Neurochemistry, 61(3), 1159-62 (English) 1993. CODEN: JONRA9. ISSN: 0022-3042.

AB Paired helical filaments, a constituent of neurofibrillary tangles in Alzheimer's disease, consist primarily of the microtubule-assocd. protein .tau.. However, the process by which the detergent-insol. filaments of the neurofibrillary tangles are formed from sol. .tau. remains unknown. Here, the authors present a potential mechanism for the abnormal aggregation of .tau. in Alzheimer's disease: the covalent crosslinking of .tau. by the enzyme ***transglutaminase*** . Macromol. complexes of .tau., formed in the presence of ***transglutaminase*** , were found to be insol. in ionic detergent, .beta.-mercaptoethanol, ***guanidine*** -HCl, and ***urea*** and, furthermore, demonstrated an increased immunoreactivity with the monoclonal antibody Alz-50. Electron microscopic studies revealed that .tau. crosslinked by ***transglutaminase*** has a defined filamentous structure. These results indicate that ***transglutaminase*** , the activity of which has been shown to increase during programmed cell death, may play a role in the formation of pathol. assocd. with Alzheimer's disease.

L6 ANSWER 38 OF 63 CAPLUS COPYRIGHT 2004 ACS on STN
1993:576165 Document No. 119:176165 Multimeric vitronectin. Identification

and characterization of conformation-dependent self-association of the adhesive protein. Stockmann, Antje; Hess, Sibylle; Declerck, Paul; Timpl, Rupert; Preissner, Klaus T. (Kerckhoff-Klin., Max-Planck-Inst. Physiol. Klin. Forsch., Bad Nauheim, D-6350, Germany). Journal of Biological Chemistry, 268(30), 22874-82 (English) 1993. CODEN: JBCHA3. ISSN: 0021-9258.

AB The adhesive glycoprotein vitronectin (VN) shows a high degree of conformational flexibility implicating that different mol. forms of the mol. may exist. Conformation-dependent monoclonal antibodies 13H1 or 16A7 that, per se, did not react with plasma VN bound to VN treated with heparin, chaotropes, detergents, pH below 6, or by heating at 56.degree.. Dependent on the stimulus, recognition of VN by these antibodies varied and preceded heparin binding and self-assocn. of VN resulting in the formation of noncovalently linked multimeric species of the protein. Both monoclonal antibodies also reacted with VN in serum or in platelet releasates as well as with VN in extracellular matrixes of endothelial cells and inhibited cell adhesion on immobilized VN. Crit. VN levels were needed for concn.-dependent multimerization indicating a nonlinear type of polymn. process. The nature of VN multimers was judged by nondenaturing gel electrophoresis, gel filtration, and sucrose gradient ultracentrifugation and revealed the formation of 3- to 16-mer multimeric species within an Mr range of 200-1200 kDa representing a mean sedimentation coeff. of 9.6 S. In electron microscopy, multimeric VN occurred as globular specimens with an av. diam. of 15-28 nm (monomeric plasma VN, 6-8 nm). In contrast to plasma VN, VN multimers were efficiently stabilized by covalent inter-mol. bonds following chem. or ***transglutaminase*** -induced crosslinking. A synthetic peptide comprising the central heparin binding region of VN (residues 348-361) not only bound to plasma VN but induces its multimerization also in plasma. During plasmin proteolysis of VN, fragments were generated that lacked the heparin binding region and that lost the ability to multimerize following ***urea*** or detergent treatment, implicating that the highly basic region is essential for multimer formation. These data suggest that non-plasma forms of VN, which are abundant in platelets and subendothelium, represent the prototype conformer of the reactive heparin binding form of VN. These findings implicate that conformationally altered forms of VN enable the adhesive protein to multimerize in a characteristic fashion and thereby endow extracellular matrix sites with unique multivalent properties.

L6 ANSWER 39 OF 63 CAPLUS COPYRIGHT 2004 ACS on STN

1993:167880 Document No. 118:167880 Solubility and hydrolyzability of films produced by ***transglutaminase*** catalytic crosslinking of whey protein. Mahmoud, Reyad; Savello, Paul A. (Dep. Nutr. Food Sci., Utah State Univ., Logan, UT, 84322-8700, USA). Journal of Dairy Science, 76(1), 29-35 (English) 1993. CODEN: JDSCAE. ISSN: 0022-0302.

AB ***Transglutaminase*** was used to crosslink covalently concd. protein solns. of .alpha.-lactalbumin and .beta.-lactoglobulin and a 1:1 (wt/wt) mixt. of these two proteins to form gels. These gels were dehydrated to produce films. Soly. of films incubated at room temp. for 24 h in buffered solvents indicated a significant relationship with glycerol concn. in the film mixt. and with the pH of the buffered solvent. The films were insol. in SDS and .beta.-mercaptoethanol. A significant relationship between soly. of all films and ***guanidine*** hydrochloride and ***urea*** in the solvent was obsd. Films incubated with the proteolytic enzymes trypsin and .alpha.-chymotrypsin produced a significant correlation between film hydrolyzability and incubation time. Utilization of ***transglutaminase*** -crosslinked whey protein as a film or food-coating material should consider the pH and the enzymic nature of the coated food surface.

L6 ANSWER 40 OF 63 CAPLUS COPYRIGHT 2004 ACS on STN

1993:75685 Document No. 118:75685 Structural and functional similarities of bovine .alpha.-crystallin and mouse small heat-shock protein. A family of chaperones. Merck, Karin B.; Groenen, Patricia J. T. A.; Voorter, Christina E. M.; De Haard-Hoekman, Willeke A.; Horwitz, Joseph; Bloemendal, Hans; De Jong, Wilfried W. (Dep. Biochem., Univ. Nijmegen, Nijmegen, 6500 HB, Neth.). Journal of Biological Chemistry, 268(2), 1046-52 (English) 1993. CODEN: JBCHA3. ISSN: 0021-9258.

AB .alpha.-Crystallin, composed of the subunits .alpha.A and .alpha.B, is a major vertebrate eye lens protein, accomplishing a structural role in

maintaining lens stability and transparency. Both subunits also occur in low amts. outside the lens, where their precise function is unknown. They are structurally related to the small heat-shock proteins (HSPs), and increasing evidence indicates that they have also functional similarities with the small HSPs. To extend the insight into these structural and functional relationships, the mouse small HSP (HSP25) was compared with bovine .alpha.-crystallin, with respect to several known properties of the latter. The .alpha.-crystallin and HSP25 resemble each other in secondary structure and have similar stability toward ***urea*** dissocn. at pH 7.0. Mixed polymers can be formed from any combination of .alpha.A-crystallin, .alpha.B-crystallin, and HSP25 subunits. Furthermore, HSP25, like .alpha.-crystallin, can function as a mol. chaperone, by suppressing heat-induced aggregation of other proteins, and is an efficient inhibitor of elastase. Finally, HSP25 is found to be a substrate for protein crosslinking by tissue-type ***transglutaminase*** , like .alpha.B-crystallin. These results thus corroborate that .alpha.-crystallin and the small HSPs have comparable functions, probably being involved in the protection of other proteins under conditions of stress.

L6 ANSWER 41 OF 63 CAPLUS COPYRIGHT 2004 ACS on STN
1991:555624 Document No. 115:155624 Polyamine-derived post-translational modification of glutamine residues in intracellular proteins. Beninati, Simone (Lab. Cell. Dev. Oncol., Natl. Inst. Dent. Res., Bethesda, MD, 20892, USA). Amino Acids: Chem., Biol. Med., [Pap. Int. Congr. Amino Acid Res.], Meeting Date 1989, 821-7. Editor(s): Lubec, Gert; Rosenthal, Gerald A. ESCOM: Leiden, Neth. (English) 1990. CODEN: 57IDAR.
AB Evidence is presented for the covalent incorporation of polyamines into glutamine-contg. proteins as the corresponding .gamma.-glutamyl adducts. This novel type of post-translational protein modification has been investigated in human integuments. It is well known that the chem. stability of the the insol. protein components of skin and hair is attributable to a high degree of .epsilon.(.gamma.-glutamyl)lysine crosslinking arising from cellular ***transglutaminase*** activity. It was found that ***urea*** -insol. fractions obtained from human callus, nail, and hair contain an addnl. type of crosslink formed through the polyamine spermidine. Further more, the precursors of the spermidine crosslinks, namely mono-(.gamma.-glutamyl)spermidines, have been identified in the ***urea*** -sol. fraction of all the integuments under investigation. Apparently, polyamines play a role in the assembly of the insol. intracellular structures of human integuments through a ***transglutaminase*** -catalyzed post-translational modification of the precursor proteins.

L6 ANSWER 42 OF 63 CAPLUS COPYRIGHT 2004 ACS on STN
1991:98888 Document No. 114:98888 Metabolic and secretory response of parotid cells to cationic amino acids. Uptake and catabolism of L-arginine and L-ornithine. Blachier, Francois; Mourtada, Ali; Gomis, Ramon; Sener, Abdullah; Malaisse, Willy J. (Lab. Exp. Med., Brussels Free Univ., Brussels, B-1000, Belg.). Biochimica et Biophysica Acta, 1091(2), 151-7 (English) 1991. CODEN: BBACAQ. ISSN: 0006-3002.
AB L-Arginine and L-ornithine, which stimulate amylase release, are taken up by rat parotid cells. L-Arginine is converted, in an NADPH-dependent manner and to a limited extent to L-citrulline in parotid cell homogenates, despite the absence of ornithine transcarbamylase activity. L-Arginine is largely converted to ***urea*** and L-ornithine. The generation of putrescine and polyamines from L-ornithine occurs at a very low rate, relative to the cell content in preformed amines. The major fate of exogenous or arginine-derived ornithine consists in its conversion to L-glutamate, which is then further metabolized. These findings raise several hypotheses for the secretory response of the parotid cells to cationic amino acids, including their accumulation as pos. charged mols. inside the cell and the generation of either NO, amines, substrates for a ***transglutaminase*** -catalyzed reaction, or ATP through oxidative catabolism. However, each of these hypotheses meets with objections, the modality for the stimulation of amylase release by cationic amino acids being eventually considered as an unsettled matter.

L6 ANSWER 43 OF 63 CAPLUS COPYRIGHT 2004 ACS on STN
1989:571562 Document No. 111:171562 Stimulus-secretion coupling of arginine-induced insulin release. Metabolism of L-arginine and

L-ornithine in pancreatic islets. Malaisse, Willy J.; Blachier, Francois; Mourtada, Ali; Camara, Javier; Albor, Amador; Valverde, Isabel; Sener, Abdullah (Lab. Exp. Med., Brussels Free Univ., Brussels, B-1000, Belg.). Biochimica et Biophysica Acta, 1013(2), 133-43 (English) 1989. CODEN: BBACAQ. ISSN: 0006-3002.

AB Exogenous L-arginine and L-ornithine rapidly accumulate in rat pancreatic islets. L-Arginine is converted to L-ornithine and ***urea*** . Endogenous or exogenous L-ornithine generates di- and polyamines, the putrescine turnover being faster than that of spermidine and spermine. However, the major pathway for L-ornithine metab. consists of its transamination to L-glutamaldehyde and further conversion to L-glutamate. The amines and L-glutamate derived from exogenous L-ornithine are incorporated into islet proteins at the intervention of ***transglutaminase*** and cycloheximide-sensitive biosynthetic processes, resp. Apparently, the insulinotropic action of L-arginine and L-ornithine could somehow be related to the metab. of these cationic amino acids in islet cells.

L6 ANSWER 44 OF 63 CAPLUS COPYRIGHT 2004 ACS on STN

1989:170940 Document No. 110:170940 Apoptotic hepatocytes become insoluble in detergents and chaotropic agents as a result of ***transglutaminase*** action. Fesus, Laszlo; Thomazy, Vilmos; Autuori, Francesco; Ceru, Maria P.; Tarcsa, Edit; Piacentini, Mauro (Dep. Biochem., Univ. Sch. Med., Debrecen, H-4012, Hung.). FEBS Letters, 245(1-2), 150-4 (English) 1989. CODEN: FEBLAL. ISSN: 0014-5793.

AB Physiol. deletion of cells ensues programmed death which involves formation of apoptotic bodies with fragmented DNA. Here it is reported that apoptotic hepatocytes are insol. in detergents, ***urea*** , ***guanidine*** -HCl, and reducing agents and thereby can be isolated from rat liver following collagenase treatment. They are wrinkled, spherical structures similar to cornified envelopes of epidermis by phase-contrast microscopy and show irregular, globular morphol. by SEM. Part of their DNA content is cleaved into nucleosomal and oligonucleosomal fragments. Their insoly., like that of the cornified envelope, is evoked by .epsilon.-(.gamma.-glutamyl)lysine and N1,N8-bis(.gamma.-glutamyl)spermidine protein crosslinking bonds formed by ***transglutaminase*** .

L6 ANSWER 45 OF 63 CAPLUS COPYRIGHT 2004 ACS on STN

1987:454391 Document No. 107:54391 .alpha.s1-Casein film prepared using ***transglutaminase*** . Motoki, Masao; Aso, Hiroshi; Seguro, Katsuya; Nio, Noriki (Cent. Res. Lab., Ajinomoto Co., Inc., Kawasaki, 210, Japan). Agricultural and Biological Chemistry, 51(4), 993-6 (English) 1987. CODEN: ABCHA6. ISSN: 0002-1369.

AB The gelation of an .alpha.s1-casein soln. with ***transglutaminase*** was applied to the prepn. of an .alpha.s1-casein film. The .alpha.s1-casein film obtained showed a high tensile strength (105 g/cm2) and strain (72%). It was insol. in water, 10% 2-mercaptoethanol, 6.6M ***urea*** , 10% SDS, and 6M ***guanidine*** HCl. Even if it was dild. with water and then heated at 100.degree. for 10 min, it remained insol. However, it was gradually hydrolyzed by chymotrypsin. These results suggest the usefulness of the .alpha.s1-casein film as a supporting material for immobilized enzymes, a medical polymer, and an edible film.

L6 ANSWER 46 OF 63 CAPLUS COPYRIGHT 2004 ACS on STN

1985:147009 Document No. 102:147009 Formation of a 55,000 molecular-weight crosslinked .beta. crystallin dimer in the calcium treated lens. A model for cataract. Lorand, Laszlo; Conrad, Sylvia M.; Velasco, Pauline T. (Dep. Biochem., Mol. Biol. Cell Biol., Northwestern Univ., Evanston, IL, 60201, USA). Biochemistry, 24(6), 1525-31 (English) 1985. CODEN: BICHAW. ISSN: 0006-2960.

AB Incubation of lens in Ca2+-contg. media, considered by several investigators to be a useful model of cataract formation and cell aging, gave rise to significant alterations in the covalent structures of various proteins. In rabbit lens, (as detected by SDS-polyacrylamide gel electrophoresis after redn. of SS bonds in ***urea***), the most readily observable changes were disappearance of 210,000-dalton (210K), 95K, and 60K proteins, modifications of .alpha.-crystallin subunits, alterations of .beta.H crystallins, and de novo prodn. of 55K and higher mol. wt. polymers. The addn. of leupeptin inhibited the disappearances of

210K, 95K, and 60K proteins and the alteration of .alpha.-crystallins, suggesting that all these were caused by a Ca2+-activated protease. The proteolytically sensitive 60K species was identified as vimentin, a component of intermediate filaments. Formation of the 55K material and of higher mol. wt. polymers during Ca2+ treatment of the lens could be prevented by histamine, a compd. known to inhibit the ***transglutaminase*** -mediated crosslinking of proteins by .epsilon.-(.gamma.-glutamyl)lysine peptide bonds in other biol. systems. It could also be shown by immunoblotting that an antibody raised against the 55K material reacted selectively with .beta.-crystallins of normal lens. This indicates that the 55K product is in all likelihood an essential dimer of certain polypeptides of .beta.-crystallin.

L6 ANSWER 47 OF 63 CAPLUS COPYRIGHT 2004 ACS on STN

1985:58396 Document No. 102:58396 Specific immobilization of an enzyme by monoclonal antibody: immobilization of guinea pig liver ***transglutaminase*** . Ikura, Koji; Okumura, Katsuzumi; Yoshikawa, Masaaki; Sasaki, Ryuzo; Chiba, Hideo (Fac. Agric., Kyoto Univ., Kyoto, 606, Japan). Journal of Applied Biochemistry, 6(4), 222-31 (English) 1984. CODEN: JABIDV. ISSN: 0161-7354.

AB A simple method for enzyme immobilization with a monoclonal antibody raised against the enzyme was studied, with guinea pig liver ***transglutaminase*** as an example. This method consists of 2 steps: a conjugation of monoclonal antibodies that bind with the enzyme without inhibitory effect on the activity with agarose gel bead supports, and immobilization of enzyme proteins onto the conjugated monoclonal antibodies through the antigen-antibody immunoreaction. The advantages of this method include (1) activity recovery after the immobilization was very high, (2) enzyme proteins in crude enzyme prepn. could be immobilized specifically, and (3) immobilized enzymes could be replaced easily by fresh enzyme proteins through a ***urea*** -buffer treatment.

L6 ANSWER 48 OF 63 CAPLUS COPYRIGHT 2004 ACS on STN

1984:405332 Document No. 101:5332 The use of high performance molecular sieving columns for the study of lymphocyte products. I. Macrophage ***transglutaminase*** interaction with products of Con A-stimulated mouse spleen cells. McEntire, J.; Oberwein, B.; Burmeister, G.; Sorg, C. (Cancer Res. Cent., Columbia, MO, 65205, USA). Journal of Liquid Chromatography, 7(5), 989-1002 (English) 1984. CODEN: JLCHD8. ISSN: 0148-3919.

AB Partially purified ***transglutaminase*** from mouse peritoneal macrophages has been prepd. and shown to utilize mouse lymphokines as substrates. Biosynthetically labeled mitogen-stimulated spleen cell products were fractionated by HPLC on a mol. sieving column. A fraction known to contain macrophage migration inhibition factor of mol. wt. 15,000 kilodaltons was reacted with the ***transglutaminase*** and rechromatographed. Higher-mol.-wt. labeled components which did not dissoc. in the presence of 6M ***guanidine*** -HCl were obsd. These data suggest that the mol. heterogeneity often reported for lymphokines may be the result of ***transglutaminase*** modification of their native structures. The relationship of these findings to possible regulatory functions in the immune response is suggested.

L6 ANSWER 49 OF 63 CAPLUS COPYRIGHT 2004 ACS on STN

1984:190410 Document No. 100:190410 ***Transglutaminase*** catalyzed crosslinking of myosin to soya protein, casein and gluten. Kurth, L.; Rogers, P. J. (Div. Food Res., CSIRO, Cannon Hill, 4170, Australia). Journal of Food Science, 49(2), 573-6, 589 (English) 1984. CODEN: JFDSAZ. ISSN: 0022-1147.

AB The covalent linkage of nonmeat proteins to myosin at temps. and pH's common in meat product processing was investigated. Iodinated proteins were crosslinked to immobilized myosin and then quantitated by gamma counting after noncross-linked material was removed by washing with 6M ***guanidine*** -HCl. Bovine plasma ***transglutaminase*** [80146-85-6] catalyzed the formation of .epsilon.-(.gamma.-glutamyl)lysine cross-links between myosin and soy protein, casein or gluten at 4-5.degree. for 16 h and at pH's from 5.5-7.0. Casein was the best substrate with .apprx.0.4 g of casein crosslinked to 1.0 g myosin.

L6 ANSWER 50 OF 63 CAPLUS COPYRIGHT 2004 ACS on STN

1984:189286 Document No. 100:189286 Comparative studies of the marginal band

and plasma membrane of the epidermis. Ogawa, Hideoki; Manabe, Motomu; Hirotani, Tetsuya; Takamori, Kenji; Hattori, Michihiro (Sch. Med., Juntendo Univ., Tokyo, Japan). Current Problems in Dermatology, 11(Norm. Abnorm. Epidermal Differ.), 265-76 (English) 1983. CODEN: APDEBX. ISSN: 0070-2064.

AB A purified membranous fraction of stratum corneum was isolated by a new method. Human stratum corneum was chopped and treated with 8M ***urea*** -50 mM Tris-HCl (pH 9.0), digested by the use of trypsin, and the product fractionated by a sucrose d. gradient to obtain sep. single cells without the cytoplasm. One sample was then treated with trypsin for 1 h and another with ***urea*** buffer for 24 h. Observations revealed a thickened inner membrane (marginal band) of .apprx.150 A. Each of the membranous samples contained a level of half-cystine markedly higher in amt. (.apprx.100 residues/1000) than in untreated samples. The marginal band also contained large nos. of glutamic acid and lysine residues, largely involved in .epsilon.-(.gamma.-glutamyl)lysine crosslinkages (.apprx.30%). To compare the membranous fraction of horny and living cells (marginal bands and plasma membranes, resp.), the fraction was then isolated from living cells. The relative amino acid compn. of the membranous fraction of the plasma membrane resembled that of human erythrocytes, but was quite different from that of the marginal band. These comparative studies of biochem. and morphol. features suggest the importance of S-S crosslinking by ***transglutaminase*** and other enzymes in the transformation mechanism of the marginal band.

L6 ANSWER 51 OF 63 CAPLUS COPYRIGHT 2004 ACS on STN

1984:154245 Document No. 100:154245 Keratolinin: the soluble substrate of epidermal ***transglutaminase*** from human and bovine tissue. Zettergren, Judy G.; Peterson, Larry L.; Wuepper, Kirk D. (Dep. Dermatol., Oregon Health Sci. Univ., Portland, OR, 97201, USA). Proceedings of the National Academy of Sciences of the United States of America, 81(1), 238-42 (English) 1984. CODEN: PNASA6. ISSN: 0027-8424.

AB Substrates of human and bovine epidermal ***transglutaminase*** (EC 2.3.2.13) were isolated and purified by ion-exchange chromatog. and preparative zone electrophoresis. These substrates of mol. wt. (Mr) 36,000, which were called keratolinin, incorporated dansylcadaverine and were pptd. by antibody. Keratolinin is ultimately polymd. on the inner leaflet of the keratinocyte membrane to form the cornified envelope. Each Mr 36,000 substrate was dissocd. by chaotropic agents or detergents into noncovalent subunits; the Mr of these subunits was 6000-6200 on electrophoresis in 15% acrylamide/1% SDS/6M ***urea*** gels. Isoelec. focusing of human or bovine keratolinin revealed 2 moieties sepd. by 0.3-0.4 pH unit (human, 5.4/5.0; bovine, 6.3/6.0). The 2 proteins were readily resolved by chromatofocusing and each isoelec. moiety of bovine keratolinin incorporated dansylcadaverine by epidermal ***transglutaminase*** and Ca and reacted with identity to antiserum to sol. Mr 36,000 keratolinin. Antiserum to human keratolinin failed to crossreact with its bovine counterpart. Antiserum to involucrin did not crossreact with either keratolinin or epidermis by immunodiffusion. Human and bovine epidermal keratolinins are biochem. similar but immunochem. distinct proteins from the epidermis. Involucrin appears only in significant quantities in cell culture.

L6 ANSWER 52 OF 63 CAPLUS COPYRIGHT 2004 ACS on STN

1982:542706 Document No. 97:142706 [3H]Fucose incorporation by healing skin wounds and the effect of ***transglutaminase*** inhibitors. Bowness, J. Michael (Fac. Med., Univ. Manitoba, Winnipeg, MB, R3E 0W3, Can.). Canadian Journal of Biochemistry, 60(8), 777-81 (English) 1982. CODEN: CJBIAE. ISSN: 0008-4018.

AB Punch wounds (3 mm) were made in the skin of rats and the animals were killed after 1 or 3 days. Plugs (4 mm) of wounded and unwounded skin were incubated in vitro with [3H]fucose. The labeled plugs were homogenized and subjected to sequential extn. with buffered salt solns., ethanol-ether, and 8 M ***urea*** - 50 mM dithiothreitol (DTT). Nondialyzable counts in the exts. and insol. residue were detd. and the incorporation of label by wounded and unwounded skin plugs was compared. Wound plugs showed a greater total incorporation of [3H]fucose. In addn., a greater proportion of [3H]fucose was found in the ***urea*** -DTT exts. The highest specific activity (dpm [3H]fucose per mg dry wt.) was found in a finely dispersed ppt., sedimenting at 10,000 .times. g but not at 100 .times. g. The ***transglutaminase*** inhibitors

aminoacetonitrile and dansyl cadaverine increased the extractability of a portion of the material which incorporated [3H]fucose without affecting the total incorporation. Thus, healing wounds have an increased biosynthetic capacity for an insol. fucosylated glycoprotein fraction and ***transglutaminase*** is necessary to make this fraction fully insol.

L6 ANSWER 53 OF 63 CAPLUS COPYRIGHT 2004 ACS on STN
1981:402558 Document No. 95:2558 Factor XIIIa-catalyzed coupling of structural proteins. Kahn, David R.; Cohen, Isaac (Rehabil. Inst. Chicago, Northwestern Univ., Chicago, IL, 60611, USA). Biochimica et Biophysica Acta, 668(3), 490-4 (English) 1981. CODEN: BBACAQ. ISSN: 0006-3002.
AB A new method was used to examine and quantitate the factor XIIIa-catalyzed coupling of various structural proteins. Proteins bound to Sepharose beads were incubated with labeled proteins in the presence of factor XIIIa, and the radioactivity of the ***urea*** -washed beads was detd. Coupling occurred between fibrin and fibronectin, fibrin and actin, myosin and fibronectin, and myosin and actin. The special importance of coupling between cytoskeletal proteins is emphasized in the light of the ubiquity of ***transglutaminases*** in cells.

L6 ANSWER 54 OF 63 CAPLUS COPYRIGHT 2004 ACS on STN
1980:124026 Document No. 92:124026 Epidermal ***transglutaminase*** . Identification and purification of a soluble substrate with studies of in vitro crosslinking. Buxman, Melodie M.; Lobitz, Cinda J.; Wuepper, Kirk D. (Health Sci. Cent., Univ. Oregon, Portland, OR, 97201, USA). Journal of Biological Chemistry, 255(3), 1200-3 (English) 1980. CODEN: JBCHA3. ISSN: 0021-9258.
AB A 36,000-mol.-wt. substrate for epidermal ***transglutaminase*** was identified immunochem. in buffer exts. of bovine snout epidermis, using antiserum to isolated high-mol.-wt.-substrate proteins recovered after crosslinking of ***transglutaminase*** . The high-mol.-wt. proteins were not present in sol. epidermal exts. in EDTA prior to crosslinking. The substrate was purified by DEAE-Sepharose CL-6B and ACA-34 gel filtration, where it demonstrated an apparent mol. wt. of 36,000. In the presence of Ca2+ and ***transglutaminase*** , the purified protein was converted to high-mol.-wt. polymers which, under conditions of high protein concn., included a protein aggregate insol. in ***urea*** , Na dodecyl sulfate, or .beta.-mercaptoethanol. Crosslinking did not occur with Ca2+ alone or in the presence of EDTA or putrescine, a competitive inhibitor. The .epsilon.-(.gamma.-glutamyl)lysine isodipeptide was identified in high-mol.-wt. products of crosslinking but not in the 36,000-mol.-wt. precursor. The intracellular assembly of the 36,000-mol.-wt. substrate, with subsequent crosslinking by ***transglutaminase*** and insolubilization into the keratinocyte membrane was postulated.

L6 ANSWER 55 OF 63 CAPLUS COPYRIGHT 2004 ACS on STN
1977:515402 Document No. 87:115402 Intermolecular cross-links in epidermal differentiation. Sugawara, Kiyoshi (Fac. Agric., Ibaraki Univ., Ibaraki, Japan). Biochem. Cutaneous Epidermal Differ., Proc. Jpn.-U. S. Semin., Meeting Date 1976, 387-97. Editor(s): Seiji, Makoto; Bernstein, I. A. Univ. Park Press: Baltimore, Md. (English) 1977. CODEN: 36DQAC.
AB .gamma.-Glutamyl-.epsilon.-lysine (I) crosslinkages in epidermal proteins were studied during differentiation of newborn rats. Five protein fractions from the epidermis were purified and their anino acid contents were analyzed following proteinase digestion. I was found in a plasma membrane fraction, in thiol-8M ***urea*** -insol., 0.5N KOH-sol. proteins, and in thiol-8M ***urea*** -sol. proteins. Incubation of 14C-labeled sol. epidermal proteins with unlabeled residual tissue in the presence of ***transglutaminase*** , the radioactivity was recovered in the thiol-8M ***urea*** insol. fraction. I crosslinks apparently have an important role in the late stages of epidermal differentiation.

L6 ANSWER 56 OF 63 CAPLUS COPYRIGHT 2004 ACS on STN
1975:589813 Document No. 83:189813 Mechanism of action of guinea pig liver ***transglutaminase*** . 10. Alkyl isocyanates as active site-directed inactivators of guinea pig liver ***transglutaminase*** . Gross, Michael; Whetzel, Norma K.; Folk, J. E. (Natl. Inst. Dent. Res., Natl. Inst. Health, Bethesda, MD, USA). Journal of Biological Chemistry, 250(19), 7693-9 (English) 1975. CODEN: JBCHA3. ISSN: 0021-9258.

AB Alkyl isocyanates were effective inactivators of guinea pig liver ***transglutaminase*** . Based on the specificity of the reaction, the protection against inactivation by glutamine substrate, and the essential nature of Ca for the inactivation reaction, it is concluded that these reagents act as amide substrate analogs and, thus, function in an active site-specific manner. Support for the contention that inactivation results from alkyl thiocarbamate ester formation through the single active site SH group of the enzyme is (a) the loss of 1 free SH group and the incorporation of 1 mol. of reagent/mol. of enzyme reaction, (b) the similarity in chem. properties of the inactive enzyme deriv. formed to those reported for another alkyl thiocarbamoylenzyme and an alkyl thiocarbamoylcysteine deriv., and (c) the finding that labeled peptides from digests of thiocarbamoyltransglutaminase and those from digests of iodoacetamide-inactivated enzyme occupy similar positions on peptide maps. ***Transglutaminase*** was inactivated neither by urethane analogs of its active ester substrates nor by ***urea*** analogs of its amide substrates. Thus, inactive carbamoylenzyme derivs. are formed only by direct addn. of the ***transglutaminase*** active site SH group to the isocyanate C:N double bond, and not, like several serine active site enzymes, by nucleophilic displacement with urethane analogs of substrate, or by nucleophilic displacement with ***urea*** analogs of substrate.

L6 ANSWER 57 OF 63 CAPLUS COPYRIGHT 2004 ACS on STN

1971:83348 Document No. 74:83348 Structural properties of guinea pig liver ***transglutaminase*** . Connellan, John M.; Chung, Soo Il; Whetzel, Norma K.; Bradley, Lynn M.; Folk, John E. (Natl. Inst. Dent. Res., Natl. Inst. Health, Bethesda, MD, USA). Journal of Biological Chemistry, 246(4), 1093-8 (English) 1971. CODEN: JBCHA3. ISSN: 0021-9258.

AB Evidence is presented that transglu-taminase (I) is composed of a single polypeptide chain of mol. wt. 80,000 to 90,000: (a) Polyacrylamide gel electrophoresis in the presence of dodecyl sulfate and mercaptoethanol gave a single band with a mobility corresponding to a mol. wt. of approx. 85,000. (b) Gel filtration in ***guanidine*** -HCl of the 14C-carb-amidomethyl carboxymethylated enzyme protein showed a single peak of absorbance and radioactivity from which a mol. wt. of approx. 85,000 was estd. (c) Amino-terminal anal. by con-ventional methods showed no evidence of free .alpha.-amino groups. A peptide, believed to contain the amino-terminal residue, was obtained by Pronase digestion and was isolated at levels of 0.75 and 0.8 mole/90,000 g of enzyme. The sequence of this peptide was detd. as pyroglutamylalanylaspartylleucine. (d) Digestion by carboxypeptidase A of the carboxymethylated enzyme protein in denaturing solvents released glycine and serine at equal rates to the level of 1 mole/90,000 g of protein. Hydrazinolysis gave approx. 1 mole of glycine. These findings, together with evidence that the enzyme protein contains 17 or 18 SH groups, but no SS bonds, form the basis for the view of an unbridged monomeric structure of I. Indication that I performs its catalytic functions in the monomeric form was obtained from the identity of the gel filtration patterns for the enzyme protein in the presence and absence of Ca2+; evidently Ca2+, which is essential for activation of I, does not affect a change in enzyme mol. wt. A revised enzyme purification procedure is presented. Rabbit antiserum against I was prepd. and used to characterize the enzyme purified by this procedure as immunol. homogeneous.

L6 ANSWER 58 OF 63 CAPLUS COPYRIGHT 2004 ACS on STN

1970:65001 Document No. 72:65001 Effect of L-asparaginase on fibrinogen and fibrin clot. Tumor growth-inhibiting reaction mechanism of L-asparaginase. Benko, S. A.; Laki, K. (Nat. Inst. Arthritis Metab. Dis., Nat. Inst. of Health, Bethesda, MD, USA). Archiv fuer Geschwulstforschung, 34(2-3), 102-15 (German) 1969. CODEN: ARGEAR. ISSN: 0003-911X.

AB L-Asparaginase (I) derived from Escherichia coli influenced the sensitivity of fibrinogen (II) against fibrin-stabilizing enzyme (III). After addn. of thrombin and III (***transglutaminase***) to the II soln. treated with I, the originating fibrin (IV) clot was more stable and more resistant to 8M ***urea*** than a IV clot arising in analogous conditions wit hout I. I released approx. 3 NH3 mols. from the II mol. probably due to hydrolysis of asparagine residues of II. It is suggested that the damaging effect of I on the IV clot is the crucial mechanism in the inhibition of tumor growth by I. Rigid IV fibers induced by the action of I are insufficient for fulfilling the role of a matrix necessary

for the formation of the stroma nourishing tumor cells.

L6 ANSWER 59 OF 63 CAPLUS COPYRIGHT 2004 ACS on STN
1970:30026 Document No. 72:30026 Effect of L-asparaginase on fibrinogen and on the fibrin coagulate (tumor-inhibitory effect of L-asparaginase). Benko, Sandor; Laki, Kalman (Szegedi Orvostud. Egyet., Szeged, Hung.). Orvosi Hetilap, 110(36), 2083-7 (Hungarian) 1969. CODEN: ORHEAG. ISSN: 0030-6002.
AB L-Asparaginase (I) from Escherichia coli altered the sensitivity of fibrinogen to the fibrin stabilizing enzyme (***transglutaminase***). When fibrinogen was pretreated with I, it yielded, on addn. of thrombin and fibrin-stabilizing enzyme, a clot that was firmer and less sol. in 8M ***urea*** than that formed from untreated fibrinogen. I released .apprx.3 moles of NH3 per mole of fibrinogen, presumably through the hydrolysis of the asparagine moieties. The inhibitory effect of I on tumor growth to a large extent may be due to its action on fibrin clot formation. The superstabilized, pathol. fibers formed from I-treated fibrinogen are less suited to serve as a matrix for the stroma and vascular bed of tumor tissue.

L6 ANSWER 60 OF 63 CAPLUS COPYRIGHT 2004 ACS on STN
1969:27008 Document No. 70:27008 Reaction mechanism of factor XIII. Loewy, Ariel G. (Haverford Coll., Haverford, PA, USA). Thrombosis et Diathesis Haemorrhagica, Supplementum, No. 28, 1-12 (German) 1968. CODEN: TDHSAF. ISSN: 0375-9997.
AB This discussion of the title topic, which includes old and new data, is concerned with the differences between sol. (sol. in a buffer soln. contg. ***urea***) and insol. (which cannot be dissolved except in solns. which break up chem. bonds) fibrin. Thrombin acts on fibrinogen and on Factor XIII, and turns this into plasma ***transglutaminase*** . This enzyme in the presence of Ca changes the soft sol. fibrin into a tough, elastic, insol. fibrin. Insol. fibrin is formed by a transglutamination reaction. In this reaction the fibrin monomers are linked. The .epsilon.-amino group of lysyl (but not asparaginyl groups) acts as donor and the amide group of glutaminyl as acceptor while NH3 is set free. Finally, .gamma.-glutaminyl-.epsilon.-aminolysine-14C (I) was mixed with a hydrolyzed mixt. of sol. and insol. fibrin, and significantly greater amts. of I were present in the insol. fibrin hydrolyzate, suggesting that in insol. fibrin 4-5 I connecting points are present.

L6 ANSWER 61 OF 63 CAPLUS COPYRIGHT 2004 ACS on STN
1968:112710 Document No. 68:112710 Enzymic control of insoluble fibrin formation. Loewy, Ariel G. (Haverford Coll., Haverford, PA, USA). Fibrinogen, 185-223 (English) 1968. CODEN: 19YKAU.
AB Insol. fibrin (I) is the normal form in clots not sol. in 5M ***urea*** as are the clots in some abnormal states. The factor (II) responsible for the formation of I (or the conversion of ***urea*** -sol. fibrin into I) has been called serum factor or fibrin-stabilizing factor, but both old and new data indicate that it might better be called plasma ***transglutaminase*** . The chem. properties of II are described. Curves are given for incorporation of glycine-14C ethyl ester (III) into bovine fibrin at different concns. of III. By use of III, levels of I could be detd. in whole plasma after removing fibrinogen (IV) by heating to 56.degree. for 3 min. and using the supernatant as a source of II. II must be activated by thrombin before it can act as an enzyme. Data are tabulated and interpreted for the release of large amts. of NH3 during I formation from various forms of IV. The blood of a patient with congenital inability to convert sol. fibrin into I also lacked II activity as measured by the incoraportion of III into casein. Expts. for detg. cross-bond formation by detecting .gamma.-glutamyl-.epsilon.-amino-lysyl branch points in I showed a difference of at least 4 branch points between sol. fibrin and I. Attempts to find .gamma.-glutamylglycine in hydrolyzates of I yielded neg. results. There appeared to be no shifts of peptide bonds between glutamine and lysine from the .alpha.- to the .gamma.-position while the expt. was in progress. Factor XIII and factor XIII complex are precursors of II. 102 references.

L6 ANSWER 62 OF 63 CAPLUS COPYRIGHT 2004 ACS on STN
1968:9615 Document No. 68:9615 Purification and properties of a fibrin crosslinking enzyme from rabbit liver. Tyler, H. M. (Univ. London, London, UK). Biochemical Journal, 104(3), 62P (English) 1967. CODEN:

BIJOAK. ISSN: 0264-6021.

AB Descriptions were given of the purification and properties of an enzyme from rabbit liver which stabilized fibrin clots (by crosslinking) but was devoid of detectable ***transglutaminase*** (I) activity and appeared to be distinct from the plasma enzyme (LL factor, FSF, factor XIII, plasma transamidase) which, in the presence of Ca2+ and thrombin, brings about the formation of a fibrin clot insol. in dil. acid or ***urea*** . The homogenates from livers, perfused until visibly free of blood and homogenized in 0.25 M sucrose contg. 5 mM EDTA, were centrifuged at high speed (80,000 g for 2 hrs.), and the stabilizing activity was purified from the supernatant soln. by pptn. at pH 5.0, extn. of the ppt. at pH 6.5 in phosphate buffer 0.05 M, and column chromatog. of the ext. on DEAE-cellulose. The final material was not homogeneous even after examn. in the anal. ultracentrifuge, but some evidence indicated the presence of aggregates. The major component with high specific activity had a sedimentation value of 3.2 S at 6.6.degree.. The partially purified material with high crosslinking activity readily incorporated glycine-1-14C Et ester into casein, and was Ca2+-dependent, heat-labile, and inhibited by low concns. of p-chloromercuribenzoate, iodoacetamide, and some primary amines. Though devoid of detectable I activity, its other properties resembled those of both the guinea pig-liver I and the plasma enzyme; unlike the latter, however, the rabbit-liver enzyme was not activated by thrombin.

L6 ANSWER 63 OF 63 CAPLUS COPYRIGHT 2004 ACS on STN

1966:466793 Document No. 65:66793 Original Reference No. 65:12473e-g Fibrin clot stabilizing enzymes from guinea pig liver. Tyler, H. M.; Laki, K. (U.S. Dept. of Health, Educ., & Welfare, Bethesda, MD). Biochemical and Biophysical Research Communications, 24(4), 506-12 (English) 1966. CODEN: BBRCA9. ISSN: 0006-291X.

AB Highly purified ***transglutaminase*** (6 .gamma. of enzyme protein) stabilized a 1 mg. fibrin clot; CBZ-L-glutaminylglycine (5 mM) or putrescine (2.5 mM) inhibited the stabilizing action of ***transglutaminase*** , presumably by competition with N- terminal glycine groups in fibrin. Highly purified ***transglutaminase*** is apparently capable of introducing cross-linkages into fibrin, presumably at the glutamine residue, causing the formation of a fibrin insol. in 8M ***urea*** . Subcellular fractionation of guinea pig liver homogenate in 0.25M sucrose indicated that the clot stabilizing activity present in this tissue remained pre- dominantly in the high speed supernatant and that, upon bringing the high speed supernatant to pH 5 and centrifuging the resultant ppt., activity was assocd. with the ppt. and could be extd. in 0.05M phosphate buffer, pH 6.5, as is known for ***transglutaminase*** . Partially purified ***transglutaminase*** was further purified by chromatography on DEAE-cellulose and the chromatograms showed the similarity between clot stabilizing activity and ***transglutaminase*** . A secondary stabilizing activity was observed on these chromatograms, suggesting that a no. of tissue transamidases exist which will cause cross-linking of fibrin monomers.

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=> S RECOMBINANT
        151943 RECOMBINANT
          6220 RECOMBINANTS
L7      155371 RECOMBINANT
                  (RECOMBINANT OR RECOMBINANTS)

=> S L7(3A)L1
L8          57 L7(3A)L1

=> S L8 NOT L6
L9          55 L8 NOT L6

=> S L7(1A)L1
L10         41 L7(1A)L1

=> S L10 NOT L6
L11         40 L10 NOT L6
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